Abstracts of the 13th International Symposium on Neural Regeneration

ORAL PRESENTATIONS

O-1 Connectomics In The Developing Nervous System

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Connectional maps of the brain may have value in developing models of both how the brain works and how it fails when subsets of neurons or synapses are missing or misconnected. Such maps might also provide the first detailed information about how brain circuits develop and age. I am especially eager to obtain such maps from the developing nervous system because of a longstanding interest in the neuromuscular circuit changes during mammalian early postnatal life. In the neuromuscular system most axonal input to muscle fibers is pruned in early postnatal life. This so called 'synapse elimination' may be part of the process whereby the nervous system molds itself to a particular epigenetic landscape. The loss is driven by competition between multiple axons that temporarily share the same junction. The amount of resources available to each axon at a particular synapse may influence the competitive outcome. Because each axon has many branches all competing roughly at the same time, the resources available at one site are likely affected by the outcome of synaptic competitions at other neuromuscular junctions that are innervated by the same axons. We have developed techniques to observe all these synaptic interactions at different sites simultaneously by computer assisted axonal tracing and the generation of transgenic mice in which different axons are labeled different colors. These *Brainbow* mice (Livet et al., 2007) give us an opportunity to see the entire connectional maps (or 'connectomes') for muscles and other neuronal circuits. Thin sectioning is required however to disambiguate the many overlapping axons. My colleagues Ken Hayworth and N. Bobby Kasthuri have developed a new kind of microtome (and an electron imaging strategy) that allows automated high resolution imaging of thousands of ultra thin (<30 nm) sections that are very large (~4 mm^2). This approach aims at making large scale serial microscopic analysis of volumes routine.

Referencess:
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O-2 Elucidating Mechanisms Of Support Offered By Skin-Derived Stem Cells In The Repair Of Peripheral Nerve Injury

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Severe injuries to peripheral nerves are common and often followed by poor functional recovery due to their chronic nature. Schwann cells (SCs) distal to the injury are crucial for successful nerve regeneration; however their capacity to maintain a growth-supportive phenotype steadily declines with prolonged denervation. In previous work, we isolated sphere-forming cells from the rodent dermis (Skin-derived Precursors, or SKPs) that showed the ability to differentiate *in vitro* and *in vivo* into GFAP/S100B positive SCs (SKP-SCs)[1]. When we used these cells in conjunction with surgical repair of a chronically denervated nerve, we observed electrophysiological and myological recovery along with improved axonal regeneration in the distal nerve of SKP-SCs treated groups significantly superior to those treated with culture media alone[2]. ELISA analysis of SKP-SC culture supernatant detected secretion of neurotrophic factors including NGF, BDNF, and NT3 in quantities surpassing that of nerve-derived SCs, and bioactive for PC12 cells. SKP-SCs also appear to elicit part of their beneficial effect via interaction with *other* cell types, since blocking host SC infiltration into SKP-SC treated injured nerve with mitomycin C impaired regeneration. Moreover, the expression of SC markers in chronically denervated nerve treated with SKP-SCs is higher than that of media treated nerve indicating that host SCs are rejuvenated by SKP-SC treatment. Gelatinase assays and Western blots confirm that SKP-SCs secrete matrix metalloproteinases, known regulators of inhibitory CSPGs present in denervated peripheral nerve. When lysate of chronically denervated nerve was treated with SKP-SC conditioned media overnight, levels of CSPGs decreased. Finaly, fresh frozen sections of chronically denervated nerve treated with SKP-SC conditioned media were rendered conducive to neurite outgrowth of rat DRG neurons versus media control. Therefore, we conclude that it is a complex combination of direct and indirect factors by which SKP-SCs elicit their beneficial effect on nerve regeneration.
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O-3 Changes In Axonal Properties During Maturation After Regeneration: A Study Using Threshold Tracking On Nerve In Vivo

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Recovery after peripheral nerve lesions with loss of axonal continuity and Wallerian degeneration is determined by the number of axons that regenerate, the specificity of reinnervation of targets, and the reestablishment of conduction properties of axons during maturation. Conduction is assumed to approach normal

since the conduction velocity in regenerated nerve has a direct relationship with the diameter of myelinated fibers, just as it is seen in normal nerve. Nonetheless, myelinated fibers have profoundly altered structure with shortened internodes and persistently reduced diameter which makes functional changes likely. We have studied cable properties and ion-channel properties *in vivo* after nerve regeneration and during degeneration in humans, cats and mice using novel threshold-tracking to study excitability of myelinated fibers, in addition to conventional nerve conduction studies and motor unit number estimation (MUNE). Excitability testing was developed by Hugh Bostock (© Institute of Neurology, Queen Square, London, UK) and allows study of the different ion-channels at the node of Ranvier and at the internode. The action potential in normal myelinated mammalian nerve fibers can be explained by activation and inactivation of Na^+-channels. However, excitability of myelinated fibers is also influenced by non-inactivating persistent Na+ channels as well as inactivating channels. The various other channels and pumps (fast and slow K+-channels, inward rectifying HCN, Na/K pump) influence excitability by stabilizing the membrane potential. These attributes can, however, not be addressed by conventional conduction studies. During degeneration and in regenerated nerves prominent changes in excitability could in part be explained by passive cable properties but in addition the changes suggested pronounced ion-channel changes in active ion-channels and the Na/K-pump og motor and sensory axons. The findings could be explained by loss of channels during degeneration and increased Na^+-load during regeneration possibly explained by increased numbers of nodes of Ranvier.

O-4 The Time Window Of Opportunity For Axon Regeneration In The Peripheral Nervous System

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The presence of the growth supportive Schwann cells in the growth pathways of the distal nerve stumps and the regenerative capacity of the motor and sensory neurons in the peripheral nervous system (PNS) have been highlighted and contrasted with the inability of axons to regenerate in the central nervous system (CNS). These have, however, masked the clinically recognized frequency of poor functional outcomes of surgical repair of injured peripheral nerves in human subjects. The poor outcomes have too often been attributed, incorrectly, to irreversible atrophy and replacement of denervated skeletal muscles and sense organs by fat. I will first briefly consider the factors that account for poor functional recovery after nerve injury and repair in the PNS of human patents. I will describe how we modeled nerve injury in animals to replicate delays in axons regenerating and reaching their denervated targets –chronic neuronal axotomy- and the chronic denervation of Schwann cells in the growth pathway during the long periods of axon regeneration at rates of 1-3mm/day. These experiments demonstrate the relatively short time window of opportunity when axon regeneration is optimal after which regenerative capacity declines progressively with time and distance. Second, I will discuss our experiments that demonstrate the effectiveness of brief electrical stimulation in accelerating axon outgrowth from the proximal nerve stumps of injured neurons and thereby, axon regeneration and target reinnervation. This brief electrical stimulation also promotes axon outgrowth in the CNS. Third, the positive and negative roles of cAMP and chondrotin sulphate

proteoglycans, respectively will be considered in PNS regeneration. Finally, I conclude considering mechanisms of the effectiveness of the electrical stimulation in promoting axon regeneration in both PNS and CNS. (Many thanks to the AHFMR and CIHR of Canada for their long-term support of our research)

O-5 Why does a lower motor neuron regenerate an axon following a peripheral nerve lesion: and how can it be persuaded to grow back to its original terminal nerve branch to muscle?

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Despite regeneration, extensive peripheral nerve injuries can result in the effective paralysis of the entire limb or distal portions of the limb. Refinement of microsurgical techniques involving the introduction of the surgical microscope and microsutures has increased the accuracy of this mechanical repair process, however only 10% of adults will recover normal nerve function using state-of-the-art techniques. The major key to recovery of function following nerve lesions in the peripheral nervous system is the accurate regeneration of axons back to their original target end-organs.

Using the rodent femoral nerve as a model system, evidence will be presented that suggests there is a hierarchy of influences that determine the accuracy of motor neuron regeneration at the level of a terminal nerve branch. Using a variety of surgical manipulations[1] and the selective removal of Schwann cells[2] (or their selective increase[3]) in the distal nerve via molecular targeting, we have examined the respective roles of end-organ influence (i.e. muscle) vs. Schwann cells in this model system. Our results suggest that regenerating motor neuron projections are not determined by inherent molecular differences between distal terminal nerve branches themselves. Rather, we propose a two-step process that shapes motor neuron reinnervation accuracy. Initial outgrowth choices made by motor axons at the transection site are proportional to the relative amount of target nerve associated with distal nerve axons that previously projected to each of the terminal nerve pathways. Secondly, the likelihood of an axon collateral from a motor neuron remaining in either terminal nerve branch is based upon the relative trophic support provided to the parent motor neuron by the competing terminal pathways and/or end-organs. Finally, we will introduce the possibility of utilizing the distal severed nerve pathway as a selective delivery mechanism to target specific Schwann cell tubes at a more proximal nerve repair site. Since the accuracy of motor neuron axon regeneration is largely determined by the Schwann cell tubes that an axon enters at this initial repair site, such anatomical targeting of specific compounds could have a significant impact on eventual regeneration accuracy.

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3. Exp. Neurol., 215, 228-235, 2009.

O-6 Micro and Nano-Scale Biomaterials That Facilitate Perpheral and Central Nerve Repair

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Injuries to peripheral and central nerves trigger a specific set of cellular and molecular responses that determines their regenerative capacity. Peripheral nerves have a limited capacity to regenerate and regenerative failure in the CNS often is due to lack of appropriate, physical, 'bridge' support that acts in concert with bodies own regenerative response. In the CNS, regeneration requires a complex, orchestrated set of events that manage and overcome local inflammation, astro-glial scar and aberrant, non productive axonal growth. Biomaterials allow for creating spatially and temporally controlled biological environments in vivo. They achieve this by taking the form of fibers, gels, nanocarriers that may or may not be degradable that are capable of incorporating biological elements such as nucleic acids, proteins and cells within them. My presentation will discuss the role of biomaterials in enhancing peripheral nerve repair and managing the complex post-injury CNS environment to create pro-regenerative environments. Oriented nanofiber based polymeric thin films create enhanced nerve guides that facilitate bridging of critically sized nerve gaps by facilitating Schwann cell migration and axonal regeneration. Data demonstrating peripheral nerve regeneration with minimal intra-luminal thin film scaffolds that occupy only 0.3% of nerve guidance channel's intraluminal volume will be presented. In the CNS, axonal growth permissive, in situ gelling hydrogels that carry polymeric nanoparticles or lipid-based microtubes, to decrease acute inflammation and digest astroglial scar tissue will be discussed. A method of thermally stabilizing the enzyme chondroitinase ABC so that polymeric sustained delivery systems can be used to obviate the need for invasive intrathecal catheters will be discussed.

O-7 Electrical Control Of Nerve Cell Behaviours

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The first report that steady, direct current (DC) electrical field stimulation promotes nerve growth and guidance appeared nearly a century ago (Ingvar, 1920). Unequivocal evidence that nerve guidance is influenced strongly by this type of electrical cue was obtained nearly thirty years ago, but there is still debate regarding the physiological significance of electrical influences on nerve growth and guidance. There is strong evidence that during nerve development and following nerve damage steady electrical signals are present naturally within tissue extracellular spaces. By mimicking these signals in culture much has been learnt regarding the mechanisms that underpin electrically directed nerve guidance. In addition, electrical signals have been applied to peripheral and central nervous system lesions in efforts to promote regeneration. Probably the most controversial and encouraging of these is the Phase 1 clinical trial in which 10 human spinal cord injured patients were exposed to DC electrical stimulation from an implanted device for 3 months and assessed neurologically at 12 months. Most showed signs of sensory improvement, but no

improvement of motor function (Shapiro et al, 2005). More recently we have shown that mammalian cortical neurones, radial glial cells and neuronal stem cells all respond directionally to steady electrical signals and that the axis of neuronal cell division is determined by a physiological electrical signal. Moving these studies in to the brain to determine how electrical signals control the cell biology of brain in development and disease is a major future challenge.

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O-8 Molecular Mechanisms Underlying Muller Glia Dedifferentiation And Retina Regeneration In Zebrafish

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Zebrafish exhibit remarkable regenerative powers and are capable of repairing a damaged retina. We recently showed that zebrafish Muller glia respond to retinal injury by dedifferentiating into a population of retinal progenitors that can regenerate all major retinal cell types (Fausett and Goldman, 2006). We also showed that the generation of these cycling progenitors depends upon the induction of the basic helix-loop-helix proneural gene, ascl1a (Fausett et al., 2008). However the mechanisms by which ascl1a regulates Muller glia dedifferentiation and proliferation are not known. Here we report that the cellular reprogramming factor, lin-28, is highly induced during retina regeneration. Following retinal injury, lin-28 is expressed in ascl1a-expressing, dedifferentiated and proliferating Muller glia. Ascl1a appears to act upstream of lin-28 since in the injured retina ascl1a knockdown blocks lin-28 induction. Lin-28 is known to suppress expression of the developmental timing miRNA, let-7, whose expression correlates with loss of self renewing cells. Indeed, let-7 expression is suppressed in proliferating Muller glia of the injured retina and lin-28 knockdown results in restoration of steady-state levels of let-7 microRNA which is accompanied by reduced cell proliferation. In conclusion, our data suggest that an ascl1a/lin-28/let-7 signaling pathway is necessary for retina regeneration by regulating Muller glia dedifferentiation and proliferation.

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O-9 Comparative Biology Of Retinal Regeneration

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Damage to the retina of fish causes the Muller glia to re-enter the mitotic cycle and generate all of the various types of retinal neurons; ie. they act as multipotent progenitors. In birds, retinal damage causes the Muller glia to proliferate, but only a subset of them give rise to new neurons, and the neurons are primarily only a single type: amacrine cells. In mice, Muller cells do not spontaneously re-enter the mitotic

cycle after retinal damage. We have investigated whether Muller glia have the potential to generate neurons in the mouse retina in vivo by eliminating ganglion and amacrine cells with intraocular NMDA injections and stimulating Muller glial to re-enter the mitotic cycle by treatment with specific growth factors. The proliferating Muller glia de-differentiate and a subset of these cells generate new amacrine cells, as defined by the expression of amacrine cell-specific markers Calretinin, NeuN, Prox1 and GAD67-GFP. These results indicate that a subset of the Muller glia in the mammalian retina have the capacity to generate new amacrine cells, like the bird, but raise the question as to what restricts their potential from also generating other types of retinal neurons. We are investigating the molecular basis for this restriction on the potential of mammalian Muller glia for complete retinal regeneration.

O-10 Use Of Human Forebrain-Derived Progenitor Cells To Stabilize Photoreceptor Degeneration And Vision Loss

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Retinal photoreceptors are highly susceptible to degeneration as a result of genetic, life style and other factors. One rational approach is to slow the progress of degenerative events, since often the associated vision loss occurs over many decades. In a series of studies we have explored the potential of transplanting cells adjacent to the photoreceptors in experimental animal models of retinal disease to achieve this goal. During the course of this work we explored the use of forebrain-derived progenitor cells, already used in a number of CNS degenerative conditions including spinal cord injury. While we had planned to use the cell as vectors for growth factor delivery, we found that untransduced cells slowed degeneration and vision loss in the RCS rat, an animal in which photoreceptors degenerate because of defect in the adjacent retinal pigment epithelium. Subsequently we found that it preserves vision in mouse models of macular degeneration, the ELOVL4 mouse and of Usher's disease, a genetic defect affecting cilia-transport functions and causing both blindness and deafness in 2 to 6.2/100,000 people. In the most common variant, Usher 2A, the affected usherin gene is large, making a gene therapy approach difficult. Like RPE65, photoreceptor loss is very slow, but functional deficits can be identified within weeks. We have found that the cell transplantation in the Usher 2A model can sustain function and reverse microanatomical changes, suggesting this condition as a particularly good target for a clinical trial. Because the cells are effective in very different mutations, not necessarily requiring cell survival or simple phagocytic role in the outer retina, they can sustain photoreceptor efficacy, overriding the effect of the causative mutation without replacing the defective gene.

O-11 Leber Congenital Amaurosis Gene Therapy Clinical Trial

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Results of a recently initiated AAV vector clinical trial for LCA2 (recessive RPE65 mutations) will be discussed. Each of three LCA2 patients received 150ul (5.9 exp10 vector genomes) of GMP grade AAV2-CBA-hRPE65 vector subretinally and their visual function then followed periodically over the next year. At 3 months post-treatment no adverse events were noted for any patient. All three also demonstrated substantial and significant improvement in light sensitivity, to 63,000 times better that their pretreatment baselines, but only in the area of retina that received vector. Upon correcting for the fraction of photoreceptors lost in each patient before treatment, we conclude that two of the three patients experienced full recovery of retinal function within the vector treated area.
At one year post-treatment, all vision improvements remained stable and no adverse events were noted. However, one patient reported new visual perceptions. When asked to detect a very bright target that she could see before treatment she continued to use her fovea, the specialized central area of the human retina. If, however, she was asked to detect a dim target that she could not previously detect at any time up to 9 months post-treatment, she could now see it. When her visual fixation was analyzed the patient shifted her center of visual perception away from her fovea into the treated retinal area. In effect, she had developed a second fovea or "pseudo-fovea" that was used only when the target was too dim to be perceived by her anatomical fovea. Determination of the retinal topography of cone sensitivity confirmed that the patient had not lost her foveal sensitivity but had gained an extra-foveal area of light sensitivity corresponding to the vector treated area. This slow emergence of a pseudo-fovea suggests that cortical "learning" is possible but slow in a young adult.

O-12 Regulation Of Stem Cell Regeneration In Skin Ectodermal Organs

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Both neural tissues and skin derive from ectoderm, While neural tissues have limited regenerative power, skin organs has robust regenerative power. This regeneration comes in the form of continuous renewal (e.g., epidermis) or episodic organ regeneration (i.e., hair and feather follicles). My laboratory has been using the skin organ paradigm to decipher the fundamental principles of morphogenesis and regeneration. Skin appendage follicles are good model because they go through regenerative cycling continuously in the adult as a physiological process. We develop the concept further: 1) organ shaping and topobiological arrangement of stem cells, 2) physiological and macro-environmental regulation of regenerative activities, and 3) re-establishing hair follicles after wound healing and periodic pattern formation. How the simple difference in topological arrangements of stem cells can determine the radial or bilateral symmetry of the organ is demonstrated with the feather model (Yue et al., 2005, 2006). How stem cell activity can be regulated by the macro-environment (subcutaneous adipose tissue, body physiological condition, and external environment) will be demonstrated with regenerative hair waves (Plikus et al., 2008). How the fundamental periodic patterning process (Chuong and Richardson edit, 2009; Maini et al., 2006) is essential for new hair formation after wounding is demonstrated by a large wound and planar hair forming model (Chuong, 2007). Finally how a

"sustainable regenerative unit" is evolved from reptile scales to hairs and feathers is discussed. To engineer stem cells into useful tissues / organs, one can learn a lot by studying how development and regeneration occur in nature, i.e. the wisdom distilled by millions of years of evolution. May be what we learn from the regeneration of skin ectodermal organs can be useful to improve the regeneration of neural ectodermal organs.

O-13 CD8+ Cytotoxic T Cells Acutely And Irreversibly Injure Demyelinated Axons In A Mouse Model Of Multiple Sclerosis

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While axon injury is a key factor in the loss of neurologic function associated with multiple sclerosis (MS), it is unclear whether damage to axons is an obligatory consequence of demyelination or whether it is an independent process that occurs in the permissive environment of the demyelinated lesion. To explicitly test the role of $CD8^+$ T cells in axon injury, we have established a perforin-deficient mouse model on an $H\text{-}2^q$ MHC background. Using the Theiler's murine encephalomyelitis picornavirus model of MS, we found that chronically infected perforin-deficient $H\text{-}2^q$ mice exhibit robust preservation of spinal axons and motor function despite the presence of severe demyelination in the spinal cord that is indistinguishable from perforin-competent animals. In contrast to previous studies into the role of $CD8^+$ T cells and perforin that used mouse strains normally resistant to TMEV infection, our observations in a susceptible MHC background directly test the hypothesis that demyelination is necessary but insufficient for axon injury by removing confounding factors related to viral biology. Using this model, we found that adoptive transfer of perforin-competent spinal cord infiltrating $CD8^+$ T cells into demyelinated but functionally preserved perforin-deficient mice led to rapid loss of motor function, disruption of spinal motor conduction, and loss of medium- and large-caliber spinal axons. These acute effector $CD8^+$ T cells were not antiviral but did exhibit a unique clonal expansion of the Vβ5.1/5.2 T cell receptor locus not observed in splenocytes or peripheral blood T cells. Our new model directly compares demyelinated lesions between perforin-competent and perforin-deficient mice, revealing that the absence of perforin protects axons without influencing demyelination. Our findings suggest that perforin is a key mediator of axon injury, lending additional support to the hypothesis that $CD8^+$ T cells are primarily responsible for the axon damage observed in MS.

O-14 Recovery Of Function Via Pathway Reorganization After Spinal Cord Injury

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Spinal cord injuries (SCI) in humans and experimental animals are often associated with varying degrees of spontaneous functional recovery during the first months after injury. Although such recovery has long been attributed to axons spared from injury that descend from the brain and bypass incomplete lesions, findings from

multiple laboratories are revealing that reorganization of descending and intrinsic (i.e. propriospinal) pathways within the spinal cord can lead to a remarkable degree of functional recovery. This presentation will review work from different laboratories, including our own, demonstrating the capacity for formation of novel relay circuits between descending supraspinal (i.e. brain) inputs and propriospinal pathways that can mediate pronounced functional recovery after severe SCI without the maintenance or regeneration of direct projections from the brain past the lesion. Such findings show that the precise restoration of point-to-point connections made by long tract-descending axons from the brain to locomotor circuits is not required to achieve meaningful functional recovery. Instead, reorganization of interactions between descending inputs and intrinsic spinal cord circuits that relay information past lesion sites can be sufficient to achieve supraspinal control of certain motor circuits. Targeting interventions to augment the remodeling of relay connections may provide new therapeutic strategies to bypass lesions and restore function following SCI and other conditions.
(Supported by NINDS, Wings for Life and Roman Reed Fund of California)

O-15 A Link Between Synaptic Plasticity and Excitotoxicity in Spinal Cord Injury

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Tumor necrosis factor-alpha (TNF) regulates glutamatergic AMPA receptor (AMPAR) trafficking in neurons (E. Beattie et al,, Science 295:2282, 2002), and appears to be a critical modulator of synaptic plasticity and stabilization. While TNF does not appear to be necessary for LTP or LTD, it now appears that in the normal brain, glutamate and TNF are in a tight regulatory balance that results in the stability of synaptic excitability via synaptic scaling (Stellwagen and Malenka, Nature, 440: 1054, 2006). Reduced neuronal activity, and thus extracellular glutamate, results in a glial release of TNFa over a period of hours. This increase in TNF mobilizes the exocytosis of GluR-2 –lacking AMPARs, increasing excitability. Spinal cord injury (SCI) and other CNS damage produces an inflammatory response that includes elaboratiron of TNF, and also releases extracellular glutamate. We have identified TNF-mediated trafficking of GluR2-lacking, Ca++-permeable AMPA receptors (CP-AMPARs) as a novel and perhaps ‘nodal’ link between injury-induced inflammation and excitotoxicity. TNF increases excitotoxic cell death in vitro, and enhances neuronal death after spinal cord injury (SCI). Further, reducing GluR2-lacking AMPAR-insertion into neuronal membranes by blocking TNFa after SCI, results in reduced neuronal death, reduced white matter damage, and better outcomes in a cervical injury model of SCI. Thus, the progression of secondary CNS injury is due, in part, to the pathophysiological ‘hijacking’ of critical synaptic regulatory mechanisms. Since TNF modulation of synaptic activity appears crucial to normal brain function, targeting TNF effects on secondary injury will require strategies that preserve that function while reducing the effects of TNF and downstream targets on excitotoxicity.
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O- 16 Learning Within The Spinal Cord

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Functional electrical stimulation and behavioral training are used to promote adaptive behavior after spinal cord injury. To further the development of these therapies, research in our laboratory has focused on developing an understanding of stimulus parameters and molecular changes that contribute to adaptive spinal plasticity. Our work suggests that controllable/predictable stimulation can induce a BDNF-dependent process that promotes adaptive plasticity. Conversely, uncontrollable/unpredictable stimulation appears to engage neurochemical systems that undermine adaptive plasticity. The capacity for learning within the isolated spinal cord is studied using rats that have undergone a thoracic transection (Grau, 2006, *Behav & Cog Neurosci Rev, 5,* 191). These spinally transected rats are given shock to one hind limb whenever that leg is extended (controllable shock). Other subjects receive the same amount of shock but independent of leg position (uncontrollable shock). Controllable shock produces a progressive increase in flexion duration, a behavioral change indicative of instrumental learning. Subjects given uncontrollable shock do not exhibit an increase in flexion duration and fail to learn when given controllable shock 24-48 hrs later (a learning deficit). Peripheral inflammation also inhibits instrumental learning. Exposure to controllable shock can both prevent, and reverse, this learning deficit. Work examining the underlying neurochemical systems has implicated the kappa opioid, NMDA and mGlu receptors, BDNF, and TNF-alpha. Studies using a contusion injury have also shown that uncontrollable, but not controllable, stimulation disrupts locomotor recovery. In addition, recent work suggests that temporal regularity can promote adaptive plasticity. Brief (80 msec) shocks given in an irregular (unpredictable) manner disable subsequent learning. In contrast, when the interval between shocks is fixed (predictable), stimulation does not have an adverse effect. Instead, it engages a BDNF-dependent process that promotes adaptive plasticity for up to 48 hrs. Supported by NS41548, HD058412, and Mission Connect.

O-17 Peripheral Inflammation Induces Acute Changes In Dorsal And Ventral Horn Glutamate Signalling

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Intraplantar carrageenan induces pain behavior, which we believe to be mediated by sensitization of sensory neurons in the dorsal spinal cord. We believe that one aspect of this sensitization is mediated by spinal tumor necrosis factor (TNF) induced increases in functional α-amino-3-hydroxyl-5-methyl-4-isoxazole-propionate (AMPA) receptors, in particular Ca2+ permeable AMPA receptors, in neuronal membranes. Our studies demonstrate that intraplantar carrageenan induces an increase in phosphorylation of Akt and GluR1 ser 845 in dorsal horn and increased

density of GluR1 and GluR4, but not GluR2 AMPA receptor subunits, in membrane fractions of dorsal horn homogenates. This change in membrane GluR1/GluR2 ratio is indicative of Ca^{2+} permeable AMPA receptor insertion into plasma membranes. Pretreatment with a TNF antagonist (Etanercept 100 μg) blocks development of these inflammation-induced biochemical changes as well as the paw carrageenan-induced pain behavior. Pretreatment with a phosphatidylinositol 3-kinase (PI-3K) antagonist (LY294002, 100 μg) had similar effects on both behavior and AMPA receptor trafficking. Pain behavior was also prevented by pretreatment with a specific antagonist to Ca^{2+} permeable AMPA receptors and Akt. The P-Akt was localized exclusively in neurons within the grey matter and oligodendrocytes within white matter. Interestingly, development of P-Akt in superficial dorsal horn and motor neurons shared a faster time course than that seen for neurons in the deep dorsal horn. Whether phosphorylation of Akt and GluR1 are in series or in parallel or upstream of AMPA receptor trafficking or pain behavior remains to be determined. Certainly, TNF mediated GluR1 trafficking appears to play a major role in inflammatory pain and TNF mediated effects such as these could represent a path by which glia contribute to neuronal sensitization (spinal long term potentiation) and pathological pain.

O-18 DEVELOPMENT OF RELIABLE AND SENSITIVE PHYSIOLOGICAL ASSESSMENTS OF OUTCOME AFTER TREATMENTS FOR SPINAL CORD INJURY

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Experimentally induced regeneration of spinal cord axons in mammals has achieved growth over a few cm at most. Assuming that similar approaches in man would not necessarily produce axonal regeneration over longer distances, protocols for assessing the outcome of such treatments would need to detect regeneration over one or two segments (Verma & Fawcett 2005, *Adv Biochem Eng Biotechnol* **94**). This would be challenging using the American Spinal Injuries Association (ASIA) standard neurological classification of spinal injuries that is currently and widely employed for clinical evaluation. The sensory and motor assessments that constitute the ASIA standard neurological classification of spinal injuries have limited sensitivity and range. Despite many revisions to the ASIA standards, including refinements such as separate upper and lower extremity motor scores (Marino & Graves 2004, *Arch Phys Med Rehabil* **85**), there remains a perceived need for sensitive, quantitative and objective outcome measures to supplement the ASIA standards clinical assessment. Initially, under a Clinical Initiative, physiological tools for improved assessment of sensory, motor and autonomic function were developed (Ellaway et al 2004, *Spinal Cord* **42**). These tools have now been tested for sensitivity and reliability against treatments expected to produce functional improvements in those with incomplete spinal cord injury (iSCI). Repetitive transcranial magnetic stimulation in cervical iSCI was employed with the aim of improving hand function and weight-assisted treadmill walking in iSCI with the aim of improving locomotion. The presentation will report on the reliability and sensitivity of a number of physiological assessments (motor, sensory and autonomic) as judged against the

functional and clinical outcomes of the treatments. The Electrical Perceptual Threshold test for cutaneous sensation, transcranial magnetic stimulation for evaluation of the corticospinal tract and the sympathetic skin response as an autonomic functional test will be evaluated (Sponsored by the International Spinal Research Trust).

O-19 BIOINFORMATICS FOR TRANSLATIONAL SPINAL CORD INJURY RESEARCH

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Spinal cord injury (SCI) produces a devastating constellation of symptoms. SCI patients experience loss of mobility as well as changes in sensory, autonomic, and fine-motor function. Animal models of SCI such as experimental hemisection, transection and contusion models have been used to study biological mechanisms regulating functional loss and recovery. Despite their apparent heterogeneity, these models produce similar symptoms that manifest across numerous outcome measures. This suggests that SCI represents a *multivariate syndrome* rather than a discrete change involving a single mechanism. However, most SCI research uses univariate statistics (e.g., t-tests, ANOVA, or bivariate correlation) that evaluate one discrete outcome measure at a time and are insensitive to patterns across multiple outcomes. These methods provide an impoverished measure of interacting SCI mechanisms and may have reduced sensitivity to experimental therapies. Multivariate approaches such as principal components analysis (PCA), partial least squares regression (PLS), multivariate analysis of variance (MANOVA), and structural equation modeling (SEM) have been used with great success in other fields with complex inter-related outcomes (e.g., epidemiology, genomics, psychology). Multivariate statistics are not commonly applied to SCI models, in part, because these methods require large datasets. To enable multivariate analysis, we are building a single large dataset from historical SCI data collected over 8 years by different research groups with shams, hemisections, and a range of unilateral contusion severities using the NYU/MASCIS and IH impactors. A large number of behavioral and histological outcomes are included. Multivariate analysis has revealed a consistent, quantifiable syndrome that varies with SCI severity and recovers over time. Early cross-species comparisons suggest that a subset of multivariate features is well-conserved, suggesting potential for quantitative translational testing of SCI therapies. Supported by R01-NS-31193, R01-NS-38079, F32-NS-053059, The Reeve Foundation, and New York State Center of Research Excellence in Spinal Cord Injury (CO- 19772).

O-20 PTPs And Vasculature As Neuroprotective Targets

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Studies in the 1960/70s proposed that vascular dysfunction could explain much of the secondary degenerative cascade after spinal cord injury. Endothelial cells are among the first cells to die at the epicenter following contusive injury and the remaining ones are dysfunctional and leaky. We are developing treatments that target these early vascular responses. Ultimately, vascular-selective drugs would allow for intravenous treatments that can rapidly and readily be applied in most clinical facilities. We have made substantial progress in mice and rats over the past few years in defining selective drug targets in endothelial cells that are amenable to therapeutic intervention. The Tie2 angiopoietin receptor and $\alpha v\beta 3$ integrin receptors are important for endothelial cell survival during development and in tumors. We have now shown in mice that i.v. injections of a combination of one of the angiopoietins and an $\alpha v\beta 3$ integrin-binding peptide reduces vascular degeneration, subsequent loss of white matter, and inflammation. The locomotor function is preserved to remarkable levels. Treatment could be initiated 4 hours post-injury and had lasting effects when terminated at 1 week. Next, we have shown in rats that inhibition of protein tyrosine phosphatases (PTPs) with the small molecule bpV(phen) rescues ascending dorsal column sensory axons and leads to normalization of related behavior and evoked potentials. Interestingly, the bpV(phen) also rescues blood vessels and the extent of rescue correlated with the extent of axonal protection. We are investigating whether the mechanism involves a vascular-specific PTP, by injecting a new selective small molecule inhibitor. We also have identified additional potential drug targets that are selectively expressed in surviving blood vessel at the epicenter, including $\alpha 1\beta 1$ integrin and ADAM8, a potential ligand for endothelial integrins or activator of pro-inflammatory cytokines. In summary, we have accelerated our progress with neuroprotective strategies after we started to focus on vascular mechanisms.

POSTER PRESENTATIONS

P-1 Gene-Specific Changes In The Structure Of Regenerating Adult Rat Retinal Ganglion Cells After Long-Term Gene Therapy

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Recombinant adeno-associated viral (AAV) vectors can be used to introduce neuroprotective genes into injured neurons, resulting in sustained supply of appropriate factors and increased neuronal viability and axonal regeneration. However it is unknown whether expression of virally produced proteins affects the morphology of transduced neurons over the long-term. This is relevant because, at least in culture, products of transgenes used to promote axonal regeneration can also alter dendritic architecture. We examined retinal ganglion cell (RGC) morphology after long-term transduction with AAV2 encoding either green fluorescent protein (GFP) or bicistronic vectors encoding GFP with ciliary neurotrophic factor (CNTF), brain-derived neurotrophic factor (BDNF) or growth-associated protein-43 (GAP43). Two weeks after intravitreal injections, rats received an autologous peripheral nerve graft onto the cut optic nerve of the AAV-injected eye. After 6-9 months, live retinas were

wholemounted and regenerating (fluorogold labelled) RGCs injected iontophoretically with 2% lucifer yellow. Dendritic morphology was documented using Neurolucida. Values were obtained for each rat RGC type (RI, RII, RIII or unclassifiable). There were no differences between saline and GFP groups in the frequency distribution of RGC types, but GFP-expressing RII cells had larger dendritic fields and were less complex. Only minor differences were seen in RGCs from AAV-GAP43-GFP injected eyes. AAV-CNTF-GFP or AAV-BDNF-GFP increased the proportion of unclassifiable RGCs to at least 50% and increased the proportion of RI and RIII relative to RII cells. In AAV-CNTF-GFP injected eyes, somas of all RGC types were significantly larger and RII cells possessed longer dendrites with greater branch complexity. After AAV-BDNF-GFP injections, RI cells were larger and had more complex dendritic branches. RII cells had increased tortuosity and larger dendritic fields, while RIII cells had longer dendrites and larger field volumes. In summary, after long-term vector transduction we have identified gene-specific changes in adult RGC morphology, changes that potentially alter the input and physiological characteristics of these neurons.

P-2 Retinal Progenitor Sheet Transplants To Rats With Retinal Degeneration – Circuitry Between Transplant And Host

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Retinal progenitor sheet transplants have been shown to restore visual responses in the brain of retinal degenerate rats, and to extend neuronal processes into the degenerated host retina. Aim of this study was to investigate the circuitry in long-term fetal retinal sheet transplants to retinal degenerate rats. - S334ter line 3 rats were transplanted at the age of 24-40d. Some donor tissues were incubated with slow-releasing microspheres containing BDNF or GDNF. Up to 265 days after surgery, eyes of selected rats were processed according to established procedures (Comp. Neurol. 464:1, 2003). Vibratome slices through the transplant area were embedded in Eponate, sectioned into serial ultrathin datasets and probed for rhodopsin, cone opsin, CRALBP (marker for Muller glia and retinal pigment epithelium = RPE), antibodies against various neurotransmitters and DAPI. - In large transplant areas, photoreceptor outer segments stained for rod and cone opsin whereas no such staining was found in the host retina. In the transplants, the photoreceptor layer thickness was inversely related to the inner plexiform layer thickness. Transplant edges and the host retina showed extensive remodeling. The transplant inner nuclear layer showed widespread loss of neurons, specifically bipolar cells, but staining for diverse amacrine cell types and horizontal cells was normal. In many areas, host and transplant neuropil was mixed together and the glial limiting membrane between the host and transplant was interrupted. The data indicate that both glycinergic and GABAergic amacrine cells are involved in the communication between transplant and host, generating alternative signal pathways between transplant and degenerating host retina. This research was supported by Lincy Foundation (MJS, RBA, HSK), NIH Grants EY02576 (RM), EY015128, EB005832 (RM), EY014800 Vision Core (RM), Research to Prevent

Blindness Career Development Award (BWJ). M.J. Seiler and R.B. Aramant have proprietary interests in the implantation instrument and procedure.

P-3 Neural Plasticity Of Primary Sensory Neurons Following Damage Of C-Type Afferents In Adult Rats

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The plasticity of the peripheral nervous system following axotomy is a well-studied phenomenon. However, changes following selective damage to non-myelinated neurons are not as well-documented. Therefore, we examined the injury-induced plasticity of primary sensory neurons following systemic administration of capsaicin, a neurotoxin selective for C- and Aδ-type afferents expressing TRPV-1. To study the effects of capsaicin in somatosensory, viscerosensory, and mixed populations of afferent neurons, we utilized the trigeminal, nodose (NG), and dorsal root ganglia. Using immunofluorescence and real-time RT-PCR, we followed the expression of TRPV-1, the NMDA receptor, and voltage-gated sodium channels (Na_v) for up to 300 days post-capsaicin. In addition, we monitored reinnervation of the viscera using an antibody against 2G13, a growth cone specific filament, and the retrograde tracer Fast Blue. As expected, TRPV-1 transcription and protein expression within sensory ganglia were dramatically decreased three days post-capsaicin, down to ~20% of vehicle. Additionally, the immunoreactivity of NR1, the obligatory NMDA receptor subunit, and NR2B were decreased by greater than 50% at this time point. The expression of $Na_v1.8$ was also reduced by ~50% at three days. However, by 60 days post-capsaicin, the number of TRPV-1 expressing neurons was not significantly different from control. NR1 expression in the NG returned to control level by 300 days. $Na_v1.8$ transcription and protein expression recovered by 60 days post-capsaicin. Furthermore, growth cones could be seen in the cervical portion of the vagus as early as 10 days post-capsaicin. By 30 days, growth cones had extended into the thoracic and abdominal levels. This was paralleled by an increase in retrogradely-labeled spinal visceral afferents from 34.6% of vehicle at 30 days to 68.9% by 60 days. These data indicate that the phenotypic alterations and loss of innervation immediately following capsaicin-induced damage may not be permanent and that sensory signaling may return with time.

P-4 The Role Of Galectin-1 in Degeneration And Regeneration Of Sensory Neurites

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Although axons in the peripheral nervous system have the ability to regrow following injury, their regenerative success is often poor. Efficient degeneration of debris in the distal nerve is necessary for proper peripheral axonal regeneration, as this debris is both a physical and molecular barrier to axonal regrowth. Interestingly, the small protein galectin-1 has been implicated in both axonal regeneration and degeneration of peripheral nerves following injury. We have shown previously that Gal1 is necessary for typical injury-induced macrophage accumulation, which has an indirect effect on the rate of nerve repair. Here, we will establish whether Gal1 affects regeneration and/or degeneration of peripheral neurites directly using cultured adult dorsal root ganglion (DRG) neurons. We and others have shown that Gal1 accelerates axonal regeneration and functional recovery after peripheral nerve injury. To study whether Gal1 effects neurite outgrowth directly, we isolated and dissociated DRG neurons and cultured them in the presence of Gal1, with or without growth factors (e.g. nerve growth factor, NGF). We found that Gal1 had no significant effect on neurite outgrowth at any of the concentrations tested. We will also establish whether Gal1 effects peripheral nerve degeneration directly. Previous work has shown that axonal degeneration is slowed in Gal1 null mutant (Lgals1-/-) mice following peripheral nerve injury. In order to determine whether this effect is direct, we will promote neurite outgrowth from dissociated DRG neurons for one day by adding growth factors (e.g. nerve growth factor, NGF) and subsequently add different concentrations of Gal1 for 1-2 days and compare neurite degeneration with positive controls (neurons cultured with vincristine). Our data indicate that Gal1 does not promote neurite outgrowth when added in the medium surrounding cultured DRG neurons. We expect that exogenous Gal1 will have a direct degenerative effect when added to previously-established neurites.

P-5 Micropatterned Adhesive Extracellular Matrix Substrates Reveal Specific Response Of Regenerating Adult DRG Neurites

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Progression of growth cones during neuronal development, or during regeneration after injury in the adult, is a step-by-step process precisely controlled by the molecular composition of the environment. To analyze local interactions of growth cones with individual molecular components *in vitro*, we created micropatterned substrates using a novel technique, micro-contact printing of spots of varying size and spacing. Here, spots were composed of laminin and/or fibronectin, extracellular matrix proteins inducing different growth cone behaviors when presented as homogeneous substrates. Using here, the paradigm of DRG neuron regeneration, we show that laminin not only stimulates neurite extension, but depending on the pattern designed, provides guidance and branching control by influencing local cytoskeletal dynamics. Indeed, mechanical measurements by Atomic Force Microscopy show that the rigidity of distal neurite segments is reinforced

through recruitment of actomyosin and modifications in the microtubule network. Observation of growth cone dynamics on a pattern alternating laminin and fibronectin spots indicates that fibronectin can constitute a transient support, facilitating exploration of a more distant (growth-supporting) environment. We propose that growth cone guidance by local factors is related to mechanical and chemical mechanisms influencing the number and strength of adhesion sites that in turn, enable the cytoskeleton to generate the forces required for neurite extension. *Supported by : CNRS, INSERM, UPMC, IRME, and BioForce Nanosciences Inc., Ames*
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P-6 Spinal Regeneration Of Sensory Axons Following Dorsal Root Rhizotomy

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Injuries to dorsal roots often lead to a major loss of sensory function and subsequent loss-of-use of the affected limb, because sensory axons cannot regenerate within the spinal cord. In recent years, some progress has been made in promoting regeneration of sensory axons into the spinal cord following dorsal root crush by blocking inhibitory influences within the cord or by stimulating more robust growth of sensory axons directly with neurotrophic factors. There has been little progress, however, in restoring sensory input to the cord when the root is completely separated from its attachment with the cord. Regenerating axons must be guided back to the spinal cord and then stimulated to grow within the cord. We describe two techniques to promote more successful regeneration of sensory axons after dorsal roots are cut rather than crushed. When lumbosacral roots were cut midway between the dorsal root ganglia and the cord, sensory axons in the distal stump could be guided back into the proximal stump by placing the ends of each stump inside a synthetic tube made of purified silk protein. Neurofilament staining revealed that rgenerated sensory axons grew through the tube and back into the distal stump, often reaching the cord. In the cervical region, because roots were too short to place in tubes, cut roots were repositioned next to the cord and held in place with fibrin glue. One month later, most cut dorsal roots had reattached to the cord. In some cases, systemic treatment with artemin promoted regeneration of these fibers within the spinal gray matter, similar to the regeneration seen after dorsal root crushes. The combination of reattachment of roots with fibrin glue coupled with treatment with artemin may therefore provide a potential therapy for the re-establishment of sensory function after root avulsions.

P-7 Stress-Mediated Enhancement of Sensory Axon Growth is Glucocorticoid and Mtor-Dependent

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Regeneration of injured axons is incomplete and results in the formation of aberrant sprouting. Although newly formed axonal arbors can form synapses, these new connections may not be advantageous. Indeed, “functional plasticity” in injured neurons is often associated with the development of spasticity and pain. Recently we showed that glucocorticoids (GCs) produced during stress have profound effects on neuronal plasticity and enhance pain-like responses. To test the hypothesis that stress and GCs promote injury-induced axonal sprouting, we measured axonal growth in sensory neurons isolated from mice exposed to acute (60 min) restraint stress immediately prior to axotomy. Ex vivo analyses showed a marked enhancing effect of stress on the magnitude of sprouting and the overall length of growing axons. The stress effects were glucocorticoid receptor (GR)-dependent. Indeed, stress-induced axon growth was significantly attenuated when stressed mice were treated with the GR antagonist (RU486) prior to stress. Also, when mice were treated with corticosterone in lieu of restraint stress, axon growth was increased. Stress activates PI3K-mTOR signaling, a pathway that is critical for protein translation and axon elongation, therefore we predicted that mTOR activation underlies increased axon sprouting by stress or GCs. Rapamycin, an mTOR inhibitor, prevented stress-induced axon growth and the stress-induced increase in phosphorylated S6, a downstream target of mTOR. Therefore, these and previous data from our lab suggest that stress and GCs, through activation of mTOR, enhance axonal sprouting and the development of pain after nerve injury.

P-8 BETA 4 Integrins: A Potential Regulator Of Chronic Nerve Compression Injury

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Chronic nerve compression (CNC) injuries, such as carpal tunnel syndrome, are the most common type of injury to the peripheral nervous system. CNC injuries are objectively distinguished by a progressive decline in nerve conduction velocity. Recent studies suggest that CNC injuries propagate through a unique injury mechanism and feature a predominantly Schwann cell mediate response. Integrins are heterodimeric mechanosensitive cell surface proteins and have been identified as potential regulators of CNC injury. The $\alpha 6\beta 4$ integrin is expressed on the abaxonal surface of mature myelinating Schwann cells and is intimately associated with the extracellular matrix. Therefore, we hypothesize that the unique cytoplasmic signaling domain of $\beta 4$ integrins is activated by mechanical stimuli and initiates the Schwann cell response to mechanical forces in CNC injury. Using an *in vivo* murine model of CNC injury, we compared the injury response in both $\beta 4$ conditional KO mice and wild type C57Bl/6 mice. Both groups were followed with weekly electrophysiology measurements for nerve conduction velocity (NCV) and amplitude. G-ratio analysis was utilized to determine the overall level of myelination at 2 and 6 week time points. Our results reveal that CNC injury induced a progressive decline in NCV in the wild type mice; however, a significantly attenuated decline in NCV was observed in $\beta 4$ conditional KO mice. Furthermore, wild type mice experienced reduced overall levels of myelination following 6 weeks of CNC injury, where as $\beta 4$ conditional KO mice did not show a significant change in myelination. Our study indicates that $\beta 4$ conditional KO mice have an attenuated response to CNC injury and $\beta 4$ integrins may

play a critical role in the pathogenesis of CNC injury. Further *in vitro* experiments with a hydrostatic pressure chamber is required to further characterize β4 integrins in CNC injury.

P-9 Localizing Integrins *IN VIVO* For Use In Regenerative Studies

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During the transition from early development to adulthood, several proteins are downregulated (or silenced), some of which are critical for the regenerative ability of neurons. Studies have shown that many integrins expressed during development are not expressed in the adult nervous system. Additionally, it has been shown that one reason why embryonic neurons are more adept at regenerating than their adult counterparts is due to an ability to increase cell surface level expression of integrins in order to grow on inhibitory substrates (Condic et al., J Neurosci, 1999). Previously, we have established that forced expression of the alpha 9 integrin subunit, the receptor for the extracellular matrix glycoprotein, tenascin-C (TN-C), significantly enhances neurite outgrowth on TN-C substrates *in vitro* and increases axon regeneration *in vivo* after either a dorsal rhizotomy or a dorsal column lesion (Andrews et al., J Neurosci. 2009). However, an important finding in our study was the difference observed between the substantial *in vitro* effect and the modest *in vivo* result. We are currently following up these results by evaluating the transport/localization of different integrin subunits *in vivo* following forced expression of a YFP-tagged integrin using lentiviral methods. Initial results suggest that integrin alpha 6-YFP (α6, receptor for laminin) remains mostly in the cell body in normal, uninjured adult rats after injection into the forelimb sensorimotor cortex with some spread into the proximal processes. However, after unilateral injection into the red nucleus, we have found a few YFP-labelled fibers in the rubrospinal tract of the cervical cord. Injuring the cervical corticospinal tract does not induce α6 transport/localization to the injury site. We are currently evaluating differences that occur with respect to distinct integrin subunit transport/localization between discrete pathways and determining whether this correlates with their regenerative capacity.

P-10 A Murine Model Of Compressive Neuropathy Defines Demyelination Distinct From Acute Nerve Injuries

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Compressive neuropathies have been considered to be variants of acute injuries and are characterized by decreased conduction velocity in myelinated fibers with minimal loss in amplitude, localized demyelination and subsequent remyelination within the zone of injury. We hypothesized that these chronic nerve compression (CNC) injuries are non-inflammatory and not primarily mediated by axonal injury with the ensuing Wallerian degeneration. We developed a novel murine

model for compressive neuropathy in wild type C57Bl/6 mice and C57BL/6J/*Wld*^{s} mice, which have delayed Wallerian degeneration. Electrophysiology recordings measuring nerve conduction velocity (NCV) were taken on a weekly basis. Morphometric analysis was performed as G-ratios from nerve cross sections and intermodal length from teased nerve fibers were calculated to assess myelination at two week and six weeks post CNC injury. Western Blot analyses of sciatic nerves for TNF alpha were performed to assess for inflammatory markers with comparison to the transection injury model. The average nerve conduction velocity (NCV) in C57Bl/6 and C57BL/6J/*Wld*^{s} mice decreased in a similar pattern over six weeks post-CNC injury with a similar corresponding increase in g-ratio, signifying a decrease in myelination. There was an absence of TNF alpha western blot expression in C57BLl/6 mice. Internodal lengths decreased over six weeks in the C57Bl/6 mice signifying that the re-myelination had occurred. These data support the hypothesis that CNC injuries are peripheral neuropathies that are not primarily mediated by inflammatory cytokines and Wallerian degeneration early after the disease. Further investigation with this novel murine model should prove to be quite useful to better understand acquired neuropathies.

P-11 Increased Voltage-Dependent NA Influx In Mouse Motor Axons Deficient Of The Myelin Protein P0

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Mice expressing half of the normal dose of the myelin protein zero (P0+/- mice) and mice completely deficient of P0 (P0-/- mice) are models for distinct forms of inherited neuropathies. P0+/- mice have almost normal myelin during the first months of life and later develop a slowly progressing demyelinating neuropathy. In contrast, P0 -/- mice display a severe neuropathy with compromised myelin compaction and axonal loss from birth.

In a previous histological study we reported that P0-deficient motor nerves may have an altered expression of Na channel isoforms, however, the extent and functional consequences of this abnormality remained unknown.

The aim of this study was to investigate in vivo the motor axon membrane function in P0 -/- and P0 +/- mice. Conventional nerve conduction studies and nerve excitability studies by “threshold-tracking” were carried out under anesthesia in 2-16 month-old P0-deficient mice. Tibial nerves were stimulated at the ankle and the evoked motor responses were recorded from the plantar muscles.

At 2 months, P0 +/- mice were undistinguishable from controls. In contrast, P0 -/- mice already showed motor responses delayed at least 200% , amplitudes reduced below 20% and marked excitability abnormalities consistent with membrane depolarization and increased voltage-dependent Na+ currents. At 16 months P0 +/- mice showed a 50% delay in motor conduction, however, the deviations in excitability measures were reminiscent of those observed in regenerated axons and were attributed to the short internodal length acquired after demyelination and remyelination.

Our data suggest that increased voltage-dependent Na+ influx in motor axons is a gain-of-function in P0 deficiency, depending on the P0 expression levels. Na-mediated axonal degeneration should therefore be considered as a potential pathogenic mechanism in inherited neuropathies with P0 mutations.

P-12 Efficient Glycomimetic Treatment Of Femoral Nerve Injury In Primates (*Macaca Fascicularis*)

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In adult mammals, restoration of function after peripheral nerve injury is often poor and efficient therapies are not available. Previously we have shown in mice that a peptide which functionally mimics the HNK-1 trisaccharide epitope significantly improves the outcome of femoral nerve injury. To evaluate this treatment in primates, we applied a linear HNK-1 mimetic or control peptide in silicone cuffs used to reconstruct the cut femoral nerves in adult cynomolgus monkeys (*Macaca fascicularis*). Using video-based analysis, we observed that gait deficits were significantly reduced in HNK-1 treated compared with control peptide treated animals between 60 to 160 days after injury. Better outcome 160 days after surgery in treated versus control animals was also confirmed by enhanced H-reflex responses, improved quadriceps muscle force and larger diameters of regenerated axons. No adverse reactions to the mimetic, in particular immune responses, were observed. These results indicate the HNK-1 mimetic as a potentially feasible, safe and efficient treatment of nerve injuries in clinical settings.

P-13 Role Of NF-kB In GFAP-Expressing Glia Following Peripheral Nerve Insult

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Schwann cells are crucial for the proper development and regeneration of axons in the peripheral nervous system. The nuclear transcription factor kB (NF-kB) was recently shown to promote Schwann cell differentiation into a myelinating phenotype *in vitro* (Yoon et al., 2008), a process required for proper axonal function. Following peripheral nerve damage, the Schwann-cell cytoskeletal protein, glial fibrillary acidic protein (GFAP), is greatly upregulated as Schwann cells dedifferentiate into an immature-like state. Based on this evidence, we tested the *hypothesis* that activation of NF-kB in GFAP-expressing glia is required for peripheral nerve regeneration. Despite functional inhibition of NF-kB in GFAP expressing glia, peripheral nerves of adult, transgenic (GFAP-IkBa-dn) mice develop

and function normally, as illustrated by normal myelin ring number, thickness and diameter. However, one month following facial nerve crush or transection, axonal regeneration is significantly reduced in GFAP-IkBa-dn mice as seen by a reduction in fluorogold labeled motoneurons in the facial motor nucleus ipsilateral to the injury. Together, these findings suggest that NF-kB activation in GFAP-expressing glia is required for efficient regeneration and functional recovery following peripheral nerve insults. [Funded by The Miami Project to Cure Paralysis]

P-14 Repair of the Deep Peroneal Nerve in the Rat by Selective Nerve Transfer of the Lateral Gastrocnemius Branch of the Tibial Nerve

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INTRODUCTION: Nerve transfer procedures show potential as a surgical alternative to traditional autologous nerve grafting. A clinically relevant rodent model of hindlimb nerve transfer has not been examined. We evaluated the effect of selective tibial branch nerve transfer on locomotor recovery, and surrogate markers of behavioural recovery, in animals following acute transection of the deep peroneal nerve. **METHODS:** Adult male Lewis rats were randomly assigned to the following treatment groups: 1) sham-operated; 2) negative control (the deep peroneal nerve and the LG branch were transected); 3) direct repair (DR) (direct deep peroneal nerve repair); and 4) neurotization (NT) (nerve transfer of the lateral gastrocnemius branch of the tibial nerve to the deep peroneal nerve). Only the right hind limb underwent surgery. Skilled locomotion was evaluated using tapered beam and ladder rung walking tasks. Flat-ground locomotion was evaluated using kinematic and ground reaction force (GRF) measures. Behaviours were evaluated preoperatively and for 2 months postoperatively. Nerve conduction velocities and muscle weights were measured 2 months postoperatively. **RESULTS:** NT-animals and DR-animals make fewer errors during skilled locomotion compared to negative-control animals but not sham-operated animals. At 2 months, GRF analysis demonstrated improvement in fore-aft forces for the forelimb ipsilateral to the affected hindlimb for the DR-group. NT and negative-control animals did not use the forelimb ipsilateral to the injured hindlimb for braking throughout the recovery period. Kinematic analysis revealed that DR-animals used their affected hindlimb similarly to sham-operated animals, though the range of motion was reduced. For the NT and negative control animals, however, the affected hock lacked extensor ability. Results of electrophysiology and muscle weight analyses supported partial reinnervation of the previously denervated cranial tibial muscle. **CONCLUSIONS:** Results suggest that the lateral gastrocnemius branch of the tibial nerve can switch its phenotype to become important during skilled but not unskilled locomotion following nerve transfer. **SUPPORT:** CIHR to RM; Robertson Fund for Cerebral Palsy Research to RM and AAW

P-15 An Aolipoprotein-E-mimetic Stimulates Axonal Regeneration and Remyelination Following Peripheral Nerve Injury

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The discovery of elevated apolipoprotein E (apoE) synthesis within the regenerating sciatic nerve advocates that apoE could benefit axon regeneration and remyelination by enhancing targeted cholesterol transport for the reconstruction of axonal and myelin membrane. Thus, apoE is considered to be a target for rationale design of therapeutic interventions to repair injured nerves. We have created an apoE-mimetic peptide, COG112, by fusing the receptor-binding domain of apoE holo-protein with a Drosophila protein transduction domain (PTD) antennapedia. We found that treatment with COG112 (1mg/kg, IP) significantly improved recovery of motor and sensory function following sciatic nerve crush in C57BL mice compared to the control groups. A morphometric analysis of sciatic nerve distal from the injured site revealed that COG112 promotes axonal regrowth as shown by increased axonal numbers and diameters after 2 weeks of treatment. More significantly, the thickness of myelin sheaths was robustly increased by COG112 treatment following injury. Consistent with these histological findings, COG112 potently elevated the gene expression and protein synthesis of growth associated protein 43 (GAP-43) and peripheral myelin protein zero (P0), which are markers of axon regeneration and remyelination, respectively. Electron microscopic examination demonstrated that there are more lipid droplet-loaded Schwann cells in sciatic nerves of COG112-treated animal than those in vehicle controls 2 weeks after injury, suggesting apoE-mimetic may increase uptake of myelin debris by Schwann cells. Consistently, we found that COG112 increased the uptake of cholesterol-containing low density lipoproteins (LDL) specifically by Schwann cells in culture. Furthermore, COG112 significantly promoted axon elongation in primary dorsal root ganglion cultures, indicating a neuroregenerative effect. Considering cholesterol supply is prerequisite for reconstruction of myelin sheaths and axon growth, these data support a hypothesis where supplementation with exogenous apoE-mimetics such as COG112 may be a promising strategy for restoring lost functional and structural elements following nerve injury.

P-16 Electrical Stimulation In Long Gap Repair: A Way To Increase Axonal And Functional Peripheral Nerve Regeneration?

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Electrical stimulation (ES) of the proximal peripheral nerve stump prior to end-to-end coaptation is known to increase preferential motor innervation and functional motor recovery. Here we investigated the effects of ES on regeneration across 13 mm peripheral nerve gaps in rats. The study evaluated three paradigms, **(I)** reconstruction using autotransplants, **(II)** transplantation of differentially filled silicone tubes and **(III)** evaluation of ES combined to gene therapy with fibroblast growth factor-2 ($FGF\text{-}2^{21/23kD}$). In paradigm **(I)** and **(II)** half of the adult female Sprague Dawley rats were kept without (w/o) ES after sciatic nerve transection as control. For ES, proximal sciatic nerve stumps were stimulated for 1h (20 Hz, 0.3mA) prior to gap reconstruction. Autotransplantation **(I)** was done by transecting and resuturing the nerve proximally and distally. Differentially filled silicone tubes **(II)**

contained: (A) matrigel alone, (B) neonatal, naïve rat Schwann cells (SC). To evaluate paradigm **(III)**, silicone tubes were filled with (C) naïve SC (n=10), (D) empty vector transfected SC, (E) SC over-expressing FGF-$2^{21/23kD}$. Functional as well as histomorphometrical analysis was performed. Two and eight weeks after surgery ES did significantly increase the nerve density at midtransplanat level and at the distal gap end, respectively, in autotransplanted sciatic nerves **(I)**. Furthermore, ES did significantly increase the nerve conduction velocity (NCV) ratio (NCV ipsilateral/contralateral) eight weeks after surgery. Analysis of experimental paradigm **(II)** revealed better macroscopic tissue regeneration through the silicone tubes in rats which received ES and transplantation of naïve SC. A combination of ES with FGF-$2^{21/23kD}$ gene therapy **(III)** did not further improve macroscopic tissue regeneration. However, this combination did result in a high rate of regenerated nerves across 13 mm gaps that did already functionally reconnect to the target muscle 8 weeks after surgery. Further evaluation will elucidate if ES did also increase precision of motor end plate reinnervation.

P-17 Deficient Recovery Of Synaptic Inputs To Motoneurons After Injury And Surgical Repair Of The Facial Nerve In Rats

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Deafferentation ("synaptic stripping") of facial motoneurons after axotomy is a well known phenomenon but it remains unknown to what degree synaptic numbers are restored after successful target reinnervation. To address this question, we performed transection and suture of the facial nerve in adult male Wistar rats. Two months after injury, a time-point at which maximal degree of functional recovery is achieved, we estimated total numbers of chemically defined synaptic terminals in the facial nucleus using stereology. We found no significant loss, compared with sham controls, of inhibitory ($VGAT^+$ GABA/glycinergic) terminals. However, both excitatory ($VGLUT2^+$ glutamatergic) and modulatory cholinergic ($ChAT^+$) terminals were significantly reduced in number, to 73% and 79% of control, respectively. Morphometric analyses revealed that while soma size of regenerated motoneurons was normal, the volume of the facial nucleus was significantly reduced (-17% compared with controls). Whether deficient functional recovery after facial nerve injury is related to the aberrant recovery of synaptic inputs is currently under investigation.

P-18 Amphetamine Enhanced Motor Training Improves Recovery Of Forelimb Function Following Cervical Contusion Injury

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Ischemic injury to the sensorimotor cortex in rats results in motor impairments, including forelimb movements used in reaching and grasping. Motor training combined with d-amphetamine (AMPH) administration has been shown to

increase dendritic arborization of cortical motor neurons, enhance monoaminergic function and ameliorate these motor deficits. A unilateral C3/4 contusion injury also produces deficits in skilled forelimb reaching and grasping. We tested the hypothesis that AMPH administration combined with motor training will enhance recovery of forelimb function through its action on descending monoaminergic pathways and task-specific training. Male rats were trained on single pellet and staircase reaching tasks six weeks before receiving the unilateral contusion injury (175 kilo dynes). Three days later, rats were performance matched and assigned to one of five groups (control, drug, training, drug + training, or drug + training + enriched environment). Motor training began 10-14 days following injury and consisted of two daily 15-minute sessions of tasks that required repetitive forelimb reaching and grasping. Forelimb function was assessed in single pellet and staircase reaching tasks. Kinematic analysis was also used to detect subtle differences that contribute to improved function. Grid walking tested locomotor function. Following sacrifice, lesion size was measured and biotin dextran amine labeled corticospinal tract neurons traced. Animals receiving combination therapy performed significantly better than control animals in the single pellet reaching task. The motor training emphasized skilled forelimb reaching and this was the function that recovered. No significant differences were observed between groups in the staircase reaching, in which grasp function is emphasized. Grid walking showed a deficit with a partial recovery but no differences between groups. Thus, this combination therapy did not transfer to behavioral tests that engage different forelimb muscles and movements. We conclude that combining AMPH with motor training increased skilled forelimb reaching function following a unilateral cervical contusion.

P-19 Chondroitinase ABC-Induced Sprouting Within The Cuneate Nucleus Of The Squirrel Monkey Promotes The Reactivation Of Denervated Somatosensory Cortex After Dorsal Column Injury

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Prior studies in rats indicate that application of chondroitinase ABC (chABC) to the dorsal column nuclei subsequent to dorsal column pathway damage, leads to functional reactive sprouting. As the effects of this brainstem-level sprouting upon denervated somatosensory cortex are unknown, we sought to determine how somatosensory cortical organization is affected subsequent to dorsal column pathway damage and chABC application to cuneate nucleus in squirrel monkeys.
A unilateral dorsal column transection at a C5/C6 level was made in 6 squirrel monkeys *(Saimiri sciureus).* This procedure resulted in the ipsilateral blockade of much of the ascending touch and proprioceptive fibers of the forelimb and digits to the cuneate nucleus. During the procedure, an ipsilateral injection of chABC or the control penicillinase enzyme was given just lateral to the cuneate nucleus. The animals were then allowed to recover. Eleven to twelve weeks later, the animals were given injections of the anterograde tracer cholera toxin B-subunit (CTB) into digits of the left and right hand. CTB-immunoreactivity within the cuneate nucleus was later used in addition to spinal cord section reconstruction to assess the extent of the dorsal column lesion.

Several days later, the somatosensory cortex contralateral to the spinal cord lesion was mapped with microelectrodes. Post-perfusion, the mapped hemisphere was separated, flattened and cut parallel to the surface. Cytochrome-oxidase staining allowed area 3b to be identified and related to the electrode penetrations made during mapping.
In the chABC-treated animals, these recordings showed that cortical territories once activated by deafferented peripheral inputs had become responsive to the afferents that remained intact after the lesion. In contrast, in animals that had not received treatment, the cortical area that had formerly received input from the deafferented periphery mostly remained unresponsive. The findings from this study indicate that chABC-induced sprouting at the level of the cuneate nucleus served to reactivate areas of somatosensory cortex that had become denervated by damage to the dorsal column pathway.

P-20 Beyond The Glial Scar: Regeneration And Targeting Of Axotomized Monoaminergic Fibres

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Regenerative treatment of spinal cord injury requires survival of neurons, regeneration of their axons beyond a glial scar, and appropriate targeting. While a great deal of work has been carried out on the former two processes, little has been done to investigate the reconnection of growing axons with target cells in the denervated cord. This is most likely due to the relatively small numbers of axons (even with more successful experimental therapies) which penetrate the distal stump of the spinal cord. As techniques to enhance regeneration across the scar improve, our attention will need to shift toward what happens on the other side. Here we assess regeneration of descending monoaminergic axons following treatment with specific neurotoxins. These have been widely used in a plethora of animal models from Parkinsonism to depression to schizophrenia, and have been useful in probing the mechanisms of nerve degeneration, regeneration, and sprouting in the central nervous system (CNS). Since neurotoxin-induced chemical damage is generally restricted to axons and their terminals, leaving cell bodies intact, the capacity for regrowth remains. While the regenerative capacities of monoaminergic neurons in the brain received much attention in the seventies and eighties, little attention has been paid since then. Further, few experiments have been carried out focusing specifically on the mammalian spinal cord. Here, we have administered neurotoxins to the thoracic spinal cord to selectively deplete serotonergic, noradrenergic, and dopaminergic populations in the adult rat using 5,7-dihydroxytryptamine (5,7-DHT), N-Ethyl-N-(2-chloroethyl)-2-bromobenzylamine hydrochloride (DSP4), and 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) respectively. We report on the differences in regenerative capacities of these three different populations of axons in the caudal spinal cord. We also examine the route that axons take towards potential targets, and provide a phenotypic characterization of targeted neurons. Understanding the differential regenerative capacities of injured axons is an essential platform on which therapeutic strategies for CNS regeneration can be investigated.

P-21 Neurotrophin– and Activity-Dependent Plasticity in the Spinal Cord Caudal to a Complete Thoracic Transection Injury

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Previous studies demonstrated the potential for intraspinal transplants of fibroblasts (Fb) genetically modified to produce neurotrophic factor (NTFs) to support robust axonal regeneration across a lesion. Hind limb exercise (Ex) is another experimental approach to increase local NTFs. Effects of these treatments on spinal cord plasticity are not clear but quantitative PCR data indicates significant change in mRNA levels for heat shock proteins, growth associated proteins and neurotrophin receptors with cycling after SCI, as possible effectors of anatomical plasticity. The present study was carried out to detect anatomical and physiological changes in the spinal cord after Fb transplantation, Ex or their combination application. A thoracic 12 (T12) complete transection lesion cavity was created in adult female Sprague-Dawley rats. Fb modified to release BDNF or GFP (as a control) were transplanted immediately after injury in 24 rats (N=12 each). Half of the animals in each group began bicycle training (60 min/day, 5d/wk) 5d post transplantation. Eight weeks later animals were subjected to EMG and H-reflex testing to assess motoneuron excitability and monosynaptic reflex activity, hind limb muscles were harvested and weighed to evaluate atrophy post SCI and animals were perfused. Horizontal sections through the transplant processed for immunocytochemical detection of neurofilaments indicated that both Fb-BDNF and Fb-GFP transplants supported axonal regeneration. For the assessment of axonal plasticity in the lumbar 4-6 spinal cord, transverse sections were reacted with antibodies to synapsin, vesicular glutamate transporter 1, vesicular glutamate transporter 2, CGRP, GAD67 or phospho-CREB. Computer assisted densitometric analysis was used to detect anatomical reorganization in the spinal cord in 3 regions (dorsal horn, intermediate gray and ventral horn) of the spinal cord. Overall, results will provide the means to correlate measures of anatomical and functional plasticity in the spinal cord after injury and application of a combination of treatment strategies. Supported by NIH Grant NS055976.

P-22 Adult Rats With A Complete Mid-Thoracic Spinal Cord Transection Benefit From Olfactory Bulb-Derived oeg Transplantation And Step Training

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Olfactory ensheathing glia (OEG) transplantation combined with treadmill step training improves hindlimb stepping in adult spinal rats (Kubasak et al., 2008). We performed a combination of behavioral, electrophysiological and anatomical evaluations on four groups of spinal rats (media-untrained and trained, OEG-untrained and trained) to better understand the mechanisms of functional recovery. We examined the maximum level of body weight support during hindlimb standing. All groups demonstrated moderate and spontaneous improvement in weight support

between 1-4 mo (P<0.001) whereas only OEG-treated rats improved between 4-7 mo (P=0.014) post-transection. Additionally, OEG-trained rats supported up to 10% of their weight with greater ankle extension than other groups at 7 mo (P<0.05). To examine sensorimotor spinal reorganization we measured the sensitivity to von Frey monofilaments using the up-down paradigm. While there were no group differences between 1-5 mo, OEG-injected rats were less hyper-reflexive than media-injected rats at 7 mo (P<0.001). Importantly, spinal cord re-transection reversed the improvements in hyper-reflexia of OEG-injected rats, but had no effect on media-injected rats. To evaluate the presence of functional reconnections across the lesion, we transcranially applied electrical stimulation to the motor cortex and brainstem. We observed motor-evoked potentials (MEPs) in hindlimb muscles in 75% of OEG-injected rats at 7 mo compared to 0% of media-injected rats. These MEPs were typically small in amplitude (0.05-0.40 mV) and long in latency (10-30 ms) compared to intact rats (n=3; 2-10 mV amplitude; 6-8 ms latency) and were lost after spinal cord re-transection rostral to the original injury site. Finally, the average volume of GFAP-negative scar and cavitations at the injury site was smaller in OEG- than media-injected rats (P=0.037). Overall, these results suggest that OEG transplantation promotes functional recovery and reconnectivity in an adult complete spinal transection model. Support: NINDS RO1 NS54159, NIH PO1 NS16333.

P-23 Combining Forced Exercise And Delivery Of BDNF And Chondroitinase Distal To A Peripheral Nerve "BRIDGE" Grafted Into A Chronic Cervical Contusion Spinal Cord Injury Site

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A peripheral nerve grafted (PNG) into a chronic cervical injury site supports robust regrowth of injured axons. Treating the distal interface with chondroitinase ABC (ChABC) allows some of these chronically injured, regenerating axons to exit the supportive environment of the graft and reenter spinal cord. We tested whether providing exogenous BDNF beyond a ChABC-treated, distal interface, would enhance the ability of axons to emerge from the graft. We grafted one end of a PN into a 4-month old unilateral, C5 contusion site. BDNF-lentivirus (Lv) or GFP-Lv was injected into C7. Two weeks later, a dorsal quadrant (DQ) lesion was made at C7. This acute injury site was treated with ChABC or PBS and the free end of the graft was inserted into the C7DQ cavity. In half of the animals, we also employed a forced exercise paradigm (Ex), which has previously been shown to increase BDNF levels and accelerate functional recovery. Thus there were a total of 8 groups: GFP-Lv+PBS-Ex, GFP-Lv+ChABC-Ex, BDNF-Lv+PBS-Ex, BDNF-Lv+ChABC-Ex, GFP-Lv+PBS+Ex, GFP-Lv+ChABC+Ex, BDNF-Lv+PBS+Ex, BDNF-Lv+ChABC+Ex. All animals were tested weekly for their ability to use their affected forelimb in the open-field, while walking on a grid, and while walking on a treadmill where specific gait parameters were measured. At the end of this long-term study, we will determine whether chronically injured axons that regenerated into the PN reformed functional synaptic contacts by electrically stimulating the PN and assessing the induction of c-fos in neurons ventral to the distal graft interface. Additionally, we will determine the extent of functional recovery that is mediated by axons within the

graft by severing the graft. We will also label axons that regrew into the graft to determine if BDNF-Lv and/or forced exercise enhanced their abilities to extend beyond the distal interface. Supported by NIH NS26380 and the Craig H. Neilsen Foundation.

P-24 Anatomical And Physiological Assessment Of Axonal Regeneration After Peripheral Nerve Grafting In Spinalized Rats

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After SCI, regeneration across the lesion site is limited due to the inhibitory environment surrounding the lesion. The combination of a peripheral nerve graft (PNG) and chondroitinase ABC (ChABC), a CSPG-digestive enzyme, provides a more permissive substrate for axonal growth leading to significant regeneration that is associated with functional recovery. Additionally, activity-dependent induction of neurotrophins, including GDNF, in the spinal cord may also provide a favorable environment for regeneration. In this study, we address the potential of a combined treatment of ChABC, GDNF and exercise on the re-growth of axons into and through a PNG and investigate potential motor recovery. A thoracic transection (T12) was performed on adult Sprague-Dawley rats (n=33), the lesion site was acutely treated with ChABC and GDNF and a predegenerated sciatic nerve inserted in the cavity. Three days later, ChABC microinjections were performed in the spinal cord caudal to the lesion. Animals were assigned to one of three groups: passive cycling (n=12), step-training (n=10) or no exercise (n=11). To assess conductivity and connectivity through the graft, magnetic motor-evoked potentials (MMEPs) were performed bi-weekly in the awake animal and the lower thoracic spinal cord was stimulated in a terminal experiment to activate c-fos. In some animals, biotinylated dextran amine was injected in the thoracic spinal cord to map propriospinal interneuron regeneration through the graft. Although no animal recovered the expression of MMEPs, immunoreaction for Neurofilament 150 identified the success of graft apposition, BDA labeled axons were found distal to the graft as were a few positive c-fos immunoreactive neurons indicating the presence of synaptic activity distal to the graft. This study demonstrates modest anatomical reconnection across a complete transection injury but indicates greater axonal outgrowth is likely necessary for any return of function. Supported by NIH grant (NS 055976) and Fonds de la Recherche en Santé du Québec.

P-25 Motor And Sensory Axon Regeneration Through A Peripheral Nerve Graft (PNG) After Thoracic Spinal Cord Injury (SCI)

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Intraspinal PNGs provide a permissible substratum for axon regeneration. Previous studies have shown regenerated axons within a PNG after a cervical injury originate mostly from supraspinal neurons but regeneration after a low thoracic injury is less well characterized. In this study we identified the source of descending and ascending axons in a PNG, their rate of extension, and neurotrophin (NT) levels within the graft to assess possible influence on overall regeneration after SCI. Adult

Sprague-Dawley rats received a T12 transection and acute PNGs, with the tibial branch of the sciatic nerve apposed to the rostral lesion wall for descending axon regeneration and the peroneal branch apposed to the caudal wall for ascending axons, and distal ends remained unapposed. Experiment 1 used a retrograde tracer, True Blue, to identify the origin of axons in the PNG. Relatively few descending supraspinal neurons (mostly from reticular formation and vestibular nucleus) were labeled in contrast to the hundreds of labeled thoracic propriospinal neurons. Ascending axon regeneration occurred from all lumbar level sensory ganglia. In experiment 2, animals were sacrificed at 1, 3, 5, 7, 10, and 17 days after grafting. Immunoreaction for Neurofilament 150 identified the leading edge of growing axons in horizontal sections through the graft. Rates of extension of ascending and descending axons will be reported. In a third experiment, NT levels in the PNG were measured by Western blotting techniques, at a time before axons entered the PNG, during active axon extension, and after axons reached the distal end. Expression of specific NTs will be correlated with the source and growth rate of axons to determine how Schwann cell-generated NTs might influence axon regeneration. This information will be useful for future experiments attempting to increase growth from the PNG distal end by NT treatment. Supported by NIH Grant (NS 055976)

P-26 The Effect Of Loose Versus Rigid Robotic Assistance On The Hindlimb During Treadmill Stepping In Spinally Contused Rats

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Robotic devices have been developed to assist treadmill training in individuals with spinal cord injuries and stroke. However, recent findings have raised questions about the effectiveness of current robotic training algorithms which rigidly enforce stepping patterns in the legs. In contrast, a “smart” robotic algorithm would only provide assistance when necessary. This type of training may be better for encouraging the intrinsic generation of movement. The purpose of this study is to examine whether robotic training that loosely controls hindlimb movement during treadmill stepping is better than enforcing a rigid pattern of movement in spinally contused rats. The loose control consists of providing an assistive force when the ankle strays from a desired trajectory. Moreover, the amount of force applied to the ankle is adjusted based on the magnitude of the movement error. For the rigid control, the ankle is moved along a fixed trajectory. 14 adult rats received a severe midthoracic spinal cord contusion (Infinite Horizon Impactor) and daily training began 1 week later (rigid group n=7; loose group n=7). EMG electrodes were implanted in the tibialis anterior and medial gastrocnemius hindlimb muscles prior to contusion. Based on on-going tests and preliminary qualitative analyses, locomotor recovery improved in both groups, however, greater EMG activity was observed in rats that were trained with the loose algorithm. Further testing and analyses will be performed to confirm these preliminary data. The findings could have important clinical implications for robotic-assisted locomotor therapies following neurological impairment.

P-27 Robotic Training With Normal Gait Patterns Influence Overground Locomotion Following Spinal Cord Injury In Rats

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In both clinical and experimental settings, repetitive training techniques have been employed to encourage neuronal plasticity and thus facilitate functional recovery following neurological injury. The recent integration of robotics into rehabilitative training allows investigators to precisely and accurately automate their training paradigms, resulting in a standardized and consistent training experience. We used a robotic gait trainer (RRMPS, Robomedica Inc, Irvine CA) to record the pre-injury normal gait pattern of 20 rats as they walked on a treadmill. Following a cervical over-hemisection injury, half of the rats received no additional training while the other half received daily robotic training where the robotic device actively guiding the injured hindlimbs through the previously recorded normal gait pattern. Over the course of the training locomotion was assessed both within the robotic device, as well as overground with the CatWalk gait analysis system (Noldus, Wageningen, NE). After four weeks of active robotic training, trained animals had significantly shorter stride lengths than the non-trained animals both within the robotic device as well as overground. Weekly locomotor assessments continued after the cessation of training. By two weeks post training, there were no longer any overground stride length differences between trained and untrained animals. These results indicate that precise and accurate robotic rehabilitative training can transiently modify overground locomotion. However, because the changes induced by training with a normal gait pattern did not lead to more normal gait, it calls into question the assumption that normal gait patterns are appropriate training patterns. There may exist a completely novel training pattern that is better suited to facilitate functional recovery following neurological injury. This work has been supported by NIH grants T32HD007459, R01NS051656, and R24HD050845.

P-28 Analysis Of Gene Expression Associated With Microvascular Activation And Neuroplasticity In Acute Activity-Based Rehabilitation Following Traumatic Rat Spinal Cord Injury (SCI).

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A promising therapeutic approach to enhancing functional recovery following SCI is the use of activity-based rehabilitation. It has been hypothesized that this intervention is most effective when initiated early after injury (i.e. 2-4 DPI) when spinal circuitry is most plastic. However, conflicting data exist regarding possible exacerbation of secondary injury cascades. Recent data from our laboratories show initiation of exercise-based rehabilitation during critical periods of post-injury microvascular plasticity exacerbates blood-spinal cord barrier (BSCB) dysfunction, possibly contributing to impaired functional recovery (Smith et al., 2009). The purpose of the current study was to provide initial data regarding transcriptional

events with early rehabilitation intervention, focusing on genes underlying enhanced microvascular dysfunction and related pathologic tissue responses. Twelve adult, female Sprague-Dawley rats received a moderate (NYU, 12.5 g/cm) T9/10 SCI. Experimental animals were then randomly assigned to exercised or non-exercised/control groups (n=6/group). Exercise consisted of intensive swim-training (2-4 x 4 minute sessions) on days 3-5 post-SCI. On day 5 post-SCI, RNA was isolated from injury epicenter tissue. Focused RT-PCR-based microarray analyses were used to examine levels of 336 mRNAs involved in regulation of microvascular plasticity, inflammation, and neurotrophin function. Of these, 55 mRNAs exhibited significant responses to swim training (≥ ± 2-fold changed). Among those implicated previously in BSCB pathology, MMP-9 and uPA were induced 5.48- and 12.46-fold, respectively. Also, several mRNAs encoding pro-inflammatory cytokines were induced by swimming, including MCP-1 (7.69-fold) and CXCL-4 (3.45-fold). Interestingly, several mRNAs associated with sensory function demonstrated robust responses to exercise (e.g. Hcrtr1-2, Galnr1-2, NpY). Fundamentally, these data identify multiple beneficial and/or detrimental systems potentiated by acute activity-based rehabilitation. Efforts are ongoing to validate and elucidate the functional consequences of this transcriptional activation by swim-training in acute SCI.
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P-29 Array Analysis Of Injured Spinal Cord Following Wheelchair Restriction Or Activity-Based Rehabilitation In The Rat

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Animal models of activity-based rehabilitation show that repetitive exposure to appropriate patterns of afferent input leads to task-specific changes in locomotor output. It has been shown that both the type and amount of afferent input can effect spinal cord function, presumably via plasticity occurring in one or more of the three functional spinal locomotor compartments; primary afferents, spinal interneurons, and/or motoneurons. We have recently developed a model of gain and loss of function based on activity-based retraining or wheelchair restriction after spinal cord injury (SCI). We hypothesized that changes in hindlimb activity post-SCI will lead to altered expression of plasticity-related molecules, giving rise to locomotor changes and functional recovery. Eighteen adult, female Sprague-Dawley rats received moderate contusive SCI at the T9 level (NYU 12.5 g/cm). Animals were randomly assigned to one of three experimental groups; swim trained starting at 7-10 days post-injury, wheelchair restricted starting at day 4 or normally housed (2 per cage). Functional recovery was monitored weekly using BBB locomotor scoring as well as the Louisville Swim Scale (LSS). At 21 days post-injury all animals were sacrificed and RNA was isolated from lumbar segments 1-3 and associated dorsal root ganglia. Significant functional deficits were evident for wheelchair restricted animals as measured by the BBB scale. RT-PCR-based microarray analysis of 172 mRNAs coding for selected ion channels, neurotransmitters and receptors showed altered expression of multiple transcripts in affected spinal tissue. Specifically, the most

robust transcriptional changes occurred for genes encoding several classes of receptors including NK1 (Taclr), gamma aminobutyric acid (GABA; B3 and A4), and acetylcholine (M3 and 4). Validation of these and identification of other primary afferent-related genes is ongoing in an effort to identify molecular signatures for activity-based rehabilitation dependent post-SCI plasticity.
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P-30 Loss And Gain Of Function: Activity-Dependent Effects After Spinal Cord Injury

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Historically, activity-based approaches to locomotor rehabilitation following spinal cord injury (SCI) have focused on models of complete transection and body-weight supported bipedal treadmill training. More recently, strategies have included more clinically relevant contusion injuries. These efforts show that repetitive exposure to appropriate patterns of afferent input can alter the capacity of the spinal cord to generate task-specific locomotor output. While small gains in overground stepping performance have been reported, most rodent studies failed to induce substantive improvement in overgound stepping beyond the already impressive functional recovery normally observed following a thoracic contusion SCI. In recent years we have used swimming and walking in shallow water as retraining strategies and observed significant task-specific functional improvements with no concomitant improvements in overground stepping. Swim-trained animals recover near normal hindlimb kinematics by 6 weeks post-injury, however hindlimb velocities remain well below normal. Shallow-water trained animals recover near normal hindlimb kinematics and dramatically improved plantar stepping, but only when the animals are assessed in shallow water, with partial weight support. We hypothesize that all these "gain of function" experiments succeed in a task-specific manner, but functional gains do not transfer to overground stepping because all experimental animals experience repetitive overground stepping during in-cage activity. We have devised a "loss of function" experiment to test this hypothesis using wheelchairs to restrict the in-cage activity experienced by rats during the first several weeks following a thoracic spinal cord injury. Kinematic analysis shows that wheelchair restriction dramatically alters the normal course of locomotor recovery and the loss of function appears to persist long after wheelchair restriction has ceased. *In toto,* these findings suggest that enhanced or restricted activity over the first few weeks post-SCI influences the course of recovery following SCI. We are now beginning to use these models to investigate the mechanisms of activity-dependent post-SCI plasticity.
This work was supported by NS052292, RR15576, and the Kentucky Spinal Cord and Head Injury Research Trust (KSCHIRT).

P-31 Fetal Serotonergic Cell Transplants Improve Ventilation After Cervical Spinal Cord Injury In Adult Rats

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Phrenic motor recovery following cervical hemisection (HS) shows a positive correlation with recovery of serotonergic input to ipsilateral phrenic motoneurons. In addition, serotonin (5-hydroxytryptamine; 5-HT) is required to initiate phrenic long-term facilitation (LTF) -- a form of motor plasticity in which persistent increases in respiratory output are observed following acute intermittent hypoxic (IH). We hypothesized that both baseline respiratory recovery and LTF expression following HS would be enhanced by intraspinal grafting of embryonic (E14) tissue derived from the dorsal raphé nuclei (DRN) and containing serotonergic neurons. Adult male Sprague-Dawley rats received HS at the caudal portion of the C2 segment followed by delayed (7 days) injection of dissociated, single-cell E14 DRN cells or vehicle-only into the C3 dorsal funiculus. Six weeks post-transplantation, minute ventilation ($\dot{V}E$) was assessed via whole-body plethysmography under normoxic baseline conditions (BL, 21% O_2), during IH challenge (3x5 min; 10% O_2) and for 60 minutes post IH to assess LTF. Under BL conditions, DRN rats (n=10) showed higher overall $\dot{V}E$ (151±21 mL/min) than sham controls (n=7; 116±47 mL/min) (p=0.05) reflecting enhanced tidal volume (TV; p=0.007). During hypoxic challenge, increases in $\dot{V}E$ were greater in DRN rats (295±46 %BL) compared to sham controls (235±64 %BL) (p=0.04) reflecting an increase in frequency (p=0.03) but not TV. LTF of $\dot{V}E$ was greater in DRN rats (150±48 %BL at 60 min) vs. sham controls (114±17 %BL, p=0.03). Histological analyses indicate that successful DRN grafts are associated with increased 5-HT immunostaining in the ventral spinal cord. These findings suggest that neurotransplantation may be a viable strategy for promoting respiratory recovery by enhancing serotonergic innervation of cervical respiratory motoneurons and possibly interneurons. **Funding:** NIH T32 HD043730 (BJD), NIH 1R01HD052682-01A1 (DDF), NIH RO1 NS054025 (PJR), and a grant from the University of Florida.

P-32 Plasticity In The Phrenic Motor System Following Contusion In Adult Rat Spinal Cord

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High cervical spinal cord injury, compromising phrenic motor pathways, results in some degree of diaphragm paresis. Such injuries often require assisted ventilation. However, experimental studies have revealed plasticity within the phrenic motor system. While previous work has focused on a partial hemisection model, less attention has been given to cervical contusion injury, which represents a more clinically relevant model of respiratory deficit post-SCI.

For the present study, adult female rats received either midline or lateralized cervical contusion injuries at C3/4 (Infinite Horizon; 150-250 kilodynes). Plethysmography was used to assess ventilation (e.g. breathing frequency and tidal volume) both prior to injury and on a weekly basis post-injury. Measurements were made under baseline (breathing normoxic, normocapnic air) and hypercapnic (7%

CO_2) conditions. All animals were then left to recover for 1-12wks post-injury. At the end of the study, measurements of diaphragm activity were obtained under terminal experimental conditions. The diaphragm was exposed and bilateral diaphragm EMG recordings were made in spontaneously breathing animals under baseline and hypercapnic conditions. Anterograde (biotin dextran amine) and transynaptic retrograde tracing (pseudorabies virus, PRV) were used to examine the changes in the phrenic circuitry following injury.

All contusion injuries resulted in substantial grey matter compromise extending into the ventral horns. Baseline ventilation was not significantly altered following injury. While the response to challenge was impaired immediately post-SCI, there was progressive recovery. In contrast, terminal diaphragm EMGs revealed significantly reduced responses to hypercapnia 12wks post-injury. Transneuronal tracing results showed increased interneuronal labeling above the site of injury. Collectively, these results suggest that compensatory mechanisms emerge post-contusion, which coincide with altered connectivity at the spinal level.
Support: Craig H. Neilson Foundation (MAL); NIH (RO1NS054025) and the Anne and Oscar Lackner Chair (PJR)

P-33 Application Of An Autologous Peripheral Nerve Bridge And Chondroitinase ABC Promotes Regeneration And Robust Recovery Of Hemidiaphragmatic Activity After C2 Hemisection

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Injuries at the cervical level that denervate the phrenic nuclei are a common type of spinal cord injury (SCI). The ensuing respiratory insufficiency, can potentially lead to death. To study these complications and strategies to promote recovery in our laboratory, we utilize the C2 hemisection (C2H) model of SCI, which results in paralysis of the ipsilateral hemidiaphragm due to the disruption of descending respiratory related bulbospinal projections. Chronic spontaneous recovery can occur via sprouting of spared fibers (e.g., serotonergic/5-HT) but only to modest levels. We have previously shown that administration of the bacterial enzyme chondroitinase ABC (ChABC) following C2H can restore an additional measure of respiratory function through degradation of inhibitory proteoglycans that limit plasticity. Other work has shown that following similar cord lesions, application of an autologous peripheral nerve bridge combined with ChABC can allow for regeneration of descending axons into and out of the graft resulting in improved forearm function. Therefore, we hypothesized that following C2H, surgical implantation of an autologous nerve bridge and treatment with ChABC could maximize re-innervation of denervated motor neurons and improve hemidiaphragmatic activity by providing both a stimulus for local sprouting as well as a regeneration bridge to bypass the lesion. The results of our experiments suggest that 12 weeks following lesion and grafting there is integration of both peripheral and central nervous system tissue, as well as substantial regeneration of axons in the peripheral nerve bridge and back into the SC. These include many different types of bulbospinal axons, including serotonergic fibers, as evidenced by TAU and 5-HT immunohistochemistry. The regeneration of these axons was reflected by the return of significantly robust hemidiaphragmatic EMG activity compared to control, non-lesioned animals and lesioned animals that

did not receive a graft. Our results show that the classic PNS bridging technique combined with ChABC can restore essentially complete function to one very important muscle.

P-34 PVPA/PVP Synthetic Film Patches As An Effective Drug Delivery System For The Acute Treatment Of Spinal Cord Injury

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A potential treatment strategy to reduce secondary damage after spinal cord injury (SCI) includes therapeutic targeting of specific components of the signaling pathways that orchestrate the inflammatory response. We previously showed that acute systemic administration of the FDA approved anti-inflammatory drug, PEGylated soluble TNF-α receptor 1 (TNF-α -R1-PEG), to injured animals during first 3 days following SCI has beneficial effect by reducing inflammatory response and secondary tissue injury. Additionally, mitogen-activated protein kinases (MAPKs) signaling pathways play crucial roles in regulating survival/cell death, neural plasticity and inflammatory responses. A preliminary study demonstrated a significant decrease in apoptosis and improved white matter sparing following intrathecal administration of p38 and JNK inhibitors. In this study we utilized a novel approach to deliver mitogen activated kinase MAPK inhibitors, SB203580, a p38 inhibitor and/or SP600125, a JNK inhibitor, and TNF-α-R1-PEG directly to the injury site using synthetic polyvinyl alcohol/polyvinyl pirolidone (PVA/PVP) film patches. *In vitro* data indicates that PVA/PVP film releases bioactive molecules by passive diffusion initially in a large bolus and then more gradually over time. Immediately following C5 unilateral cervical contusion, drug impregnated PVA/PVP patches were positioned subdurally directly on the surface of the injured rat spinal cord encompassing the C4-C6 region. Twenty four hours later, fresh spinal cord tissue extracts from the lesion epicenter and the adjacent caudal region were prepared for protein analysis. Using quantitative Western blot analysis, we found a significant reduction in the expression of phosphorylated MAPKAP2 and JNK and caspase 3 after application of MAPK inhibitors patches and heat shock proteins (HSPs) after TNF-a-R1-PEG patches compared to vehicle control. Our results indicate that PVA/PVP films are an effective drug delivery system for acute treatment after SCI with potential long term beneficial effect attenuating secondary damage after the injury.
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P-35 Fibrin-Based Scaffolds For Neural Progenitor Cell Transplantation After Spinal Cord Injury

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The purpose of this study was to evaluate the effect of controlled growth factor delivery from fibrin scaffolds on embryonic stem cell derived neural progenitor cells (ESNPCs) transplanted after a sub-acute (2 week) rat spinal cord injury (SCI). In previous *in vitro* studies, fibrin scaffolds modified with a heparin-binding delivery system (HBDS), neurotrophin 3and PDGF-AA promoted the survival and differentiation of embedded ESNPCs into neurons (~45%) and oligodendrocytes (~50%). Based on these results, the delivery of these two growth factors (GFs) from fibrin scaffolds in combination with ESNPCs was evaluated *in vivo* following SCI. A dorsal hemisection SCI was made at thoracic level 8 of Long Evans rats. Two weeks after the initial injury, the lesion site was re-exposed, and a fibrin scaffold (+/- HBDS and GF) containing ESNPCs or ESNPCs alone (control) were implanted into the lesion. At 2 weeks after transplantation, fibrin scaffolds with GFs (+/-HBDS) showed an increase in cell number of transplanted cells by stereology compared to fibrin alone or no scaffold, however only the group with the HBDS demonstrated an increase in the number of ESNPC-derived neurons (NeuN+) by stereology. At 4 weeks, all groups receiving ESNPCs showed an improvement in functional recovery as assessed by a grid walk test (decreased fraction of foot falls) compared to untreated injured controls. These results demonstrate that the combinations of fibrin scaffolds and GFs can be used to enhance ESNPC survival and differentiation after sub-acute SCI, and functional recovery can also be improved with the use of ESNPCs.

P-36 Responses Of A Unique Population Of Spinal Cord Neurons To White Matter Degeneration

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We generally think of spinal grey and white matter as distinct functional regions: grey matter contains neuronal somata and synapses, whereas white matter is made up of axonal tracts. This is an oversimplification as many spinal neurons extend dendrites for long distances into white matter where they receive synaptic input from a variety of sources. Spinal cord injury results in the degeneration of long axon tracts, which has the potential to adversely affect the structure and function of dendrites contained therein. There exists a unique population of neurons in the gracile fasciculus which is ideally suited to the study of myelin degeneration on dendritic structure. These are most frequent in spinal enlargements, and send highly varicose dendrites to the pial surface and to the central grey matter. Neurochemically and functionally, they resemble lamina III-V wide-dynamic range neurons. They are widely-separated, and thus particularly amenable to confocal microscopic analysis of somal shape and dendritic architecture. Here we investigate responses of cervical gracile fasciculus neurons to spinal cord injury. Rats received complete spinal cord transection at the level of the third thoracic vertebra and were allowed to survive for one week, one month or three months. Parasaggital sections of cervical enlargements were processed via floating section immunohistochemistry for MAP2 (to visualize somata and dendrites) and NK1 (to label a subpopulation of gracile fasciculus neurons potentially involved in nociceptive signaling). Sections were also labeled with antibodies against descending (serotonergic and noradrenergic), intrinsic (interneuronal) and primary afferent (CGRP, VGLUT1) axons. In intact animals, these targed separate compartments of gracile fascicuclus neurons, and in this work

we report on compartmental changes associated with degeneration of en-passant axons. This work allows for a better understanding of the effects of secondary damage on neuronal structure, and provides a platform upon which to study experimental neuroprotective strategies.

P-37 Transplantation Of Human PDGF-Responsive Precursors (PRPs) Into The Injured Rat Spinal Cord To Promote Remyelination Of Spared Axons

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Remyelinating strategies have gained popularity as a potential treatment for spinal cord injury (SCI) due in large part to evidence of widespread oligodendrocyte death and long-term white matter defects following injury. One such strategy currently under investigation is the use of cell replacement as a means to improve remyelination following SCI. Several investigators have found benefits to such a strategy, however, the optimal cell for transplantation to treat SCI remains to be determined. We have isolated platelet-derived growth factor (PDGF)-responsive neural precursors (PRPs) from fetal human forebrain. Compared to the commonly used human epidermal growth factor (EGF) or fibroblast growth factor (FGF)-responsive neural precursors, we found that human PRPs displayed a more robust potential to make oligodendrocytes *in vitro*. Therefore, we tested the potential of human PRPs to remyelinate axons in a rodent thoracic contusion injury model. One week following injury, human PRPs were transplanted rostral and caudal to the lesion site. Subsequent histological examination at five and nine weeks post-transplant demonstrated that human PRPs were able to survive and integrate well into host tissue. Many transplanted human PRPs co-labelled with the oligodendrocyte precursor markers NG2 and PDGF receptor-alpha as well as the premyelinating oligodendrocyte marker CNPase. Using a human mitochondrial specific marker, we also found human specific processes in close association with the mature myelin marker MBP, providing evidence of remyelination by the transplanted cells. These findings suggest that human PRPs represent a source of myelin for white matter repair after SCI, which makes these cells suitable for further study aimed at developing potential clinical applications. This research is supported by the Stem Cell Network and CIHR.

P-38 Axon Regeneration In The Injured Newt Spinal Cord

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Unlike adult mammals, adult newts are able to functionally recover after a spinal cord injury. This recovery requires severed axons to regenerate through the injury site and make functional connections with downstream targets. Using an axon tracer, we characterized stages of axon regeneration through a complete transection injury. Descending and ascending axons from the rostral and caudal stumps,

respectively, appear to go through similar stages. In the first week, axons appear to have retracted from the ends of the cut cord and to have become dystrophic. By the end of the first week, axons initiate growth and grow back to the end of the cut cord. As axons continue to grow, they often wrap around the end of the sealed central canal before wisping out ahead of the central canal into the injury site. Such wisping axons, however, rarely appear to wander into the injury site alone, but rather, remain associated with cells and cell processes. Analyses with antibodies against several extracellular matrix (ECM) molecules suggest that the cells associated with wisping axons may be meningeal or endothelial cells as such cells appear to express the same ECM molecules in the intact spinal cord. The cell processes associated with wisping axons are immunoreactive for antibodies against two proteins found in ependymal cell and astrocytic processes in the intact newt spinal cord, namely glial fibrillary acidic protein and glutamine synthetase. These observations suggest that there may be a synergistic relationship between regenerating axons and a variety of cell types that enables successful regeneration. These results shed light on how nature has solved the problem of spinal cord regeneration in an adult vertebrate and may provide insights into how to improve spinal cord regeneration in mammals.

P-39 Cellular And Molecular Characterization Of Neural Regeneration In Planarians

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Fresh water planarians posses remarkable regeneration capacity that allow repair or replacement of any injured or missing part of the nervous system in a short period of time. For example, a decapitated planarian can regenerate an entire brain within 10 days. Recent advances in cellular and molecular techniques applicable to the planarian *Schmidtea mediterranea*, including the completion of the *S. mediterranea* genome sequence and the ability to perform large scale RNAi screens, provide a great opportunity for study of neural regeneration using planarians. Here we report the characterization of cellular and molecular mechanisms controlling axon repair and neuronal replacement. Using lipophilic dye labeling and in vivo imaging, we observed that severed axons are repaired via axon fusion or axon regrowth within 24 hours. Axon fusion requires presence of the neuronal cell body of injured axons but not presence of a totipotent adult stem cell population, the neoblasts. In addition, in cases where axon regrowth takes place, we observed that a newly extended axon appears to follow the old distal fragment. We are further characterizing the cellular requirements for these two processes and using RNAi to test the molecular requirements for axon fusion and/or axon regrowth. By performing microarray analyses to identify genes with expression up-regulated during brain regeneration, we identified a homeobox gene PBX (*P*re-*B* Cell Homeobo*x*). *PBX(RNAi)* animals fail to produce photoreceptor neurons during regeneration and gradually lost these cells during homeostasis, suggesting that PBX is required for replacement of the photoreceptor cells. *PBX(RNAi)* animals are also uncoordinated and we are currently characterizing the underlying cellular defects to further our understanding of the function of PBX during neuronal replacement.

P-40 Three-Dimensional Imaging Of The Entire Spinal Cord For Assessing Axon Regeneration And Glial Responses After Injury

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We describe a method for imaging neuronal and non-neuronal cells in unsectioned adult rodent CNS tissue, including heavily myelinated spinal cord. We developed a clearing procedure that renders adult CNS tissue transparent and used 'ultramicroscopy' to image this tissue three-dimensionally. Neurons and glial cells labeled with GFP or synthetic fluorescent dyes were visualized in the entire spinal cord without histological sectioning using this approach. This allowed us to follow the complete trajectories of regenerating axons through the white and gray matter within the complete spinal cord. Moreover, the density and spatial distribution of microglia and astrocytes were determined precisely in the lesioned spinal cord. Thus, three-dimensional imaging enables unequivocal evaluation of axon regeneration and glial reactions after spinal cord injury.

P-41 Maturation Of Motor Neuron Progenitors Derived From Human Embryonic Stem Cells For Use In A High-Throughput Assay For ALS Target Discovery

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Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease that affects motor neurons (MNs) of the central nervous system that to date lacks an effective therapy. Increased levels of fasting glutamate has been detected in the serum and plasma of people with ALS, likely contributing to the neuronal loss as MNs are particularly susceptible to calcium dependent glutamate toxicity. As part of an ALS Association-sponsored project (TREAT-ALS), we aim to develop a high-throughput assay for ALS target discovery. Human embryonic stem cell (hESC) derived motor neuron progenitors (MNPs) were matured in differing media formulations to determine the optimal conditions to yield matured human MNs. Matured MNs were further characterized for expression of MN specific markers, glutamate toxicity, and electrophysiological activity. Immunocytochemical characterization showed that cultures were positive for MN markers HB9, Islet-1,

ChAT and SMI32. Matured MNs were tested for toxicity following exposure to glutamate. Exposing matured MNs to increasing concentrations of glutamate for 24 and 72 hours resulted in toxicity. Interestingly, addition of trophic factors to the media during the maturation period reduced the percent of glutamate toxicity, demonstrating that growth factor withdrawal assays can also be developed for drug discovery screening. Moreover, we examined the electrophysiological properties of the matured MNs to characterize the *in vitro* functionality of the MNs. Functional characterization of the matured MNs via whole-cell patch-clamp electrophysiology resulted in the demonstration of spontaneous synaptic activity as well as evoked action potentials. Furthermore, we observed tetrodotoxin-sensitive inward Na+-currents and outward K+-currents with varied kinectics. In the majority of recordings, exposure to GABA evoked inward currents. Additionally, inward currents were also observed upon application of glutamate. Taken together, these data indicate that hESC-derived MNs can be cultured in high throughput screening format. Immunocytochemical and electrophysiological characterization, accompanied by glutamate sensitivity confirmed that the cells closely resemble MNs.

P-42 The Effects Of Human Motor Neuron Progenitor Transplants In A Mouse Model Of Spinal Muscular Atrophy

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Infantile spinal muscular atrophy (SMA) is the most common and severe hereditary neurological disease in childhood, and is characterized by motor neuron loss. Children diagnosed with SMA demonstrate severe muscle weakness that makes it difficult for them to breathe, eat, and move, and >95% die within 2 years. There is currently no treatment that can change the course of the disease. This study set out to determine the histological, molecular, and functional effects following intraspinal transplantation of human embryonic stem cell-derived motor neuron progenitors (hESC-MNPs) into established models of Δ7SMA (*SMNdelta7;SMN2;Smn-/-),* and ALS (G93A SOD1*),* both of which are characterized by motor neuron loss. hESC-MNPs were transplanted into both models and their migration, engraftment, differentiation and repair potential within the diseased or injured tissue was assessed. Our data demonstrates limited migration, stable engraftment, differentiation, sparing of endogenous tissue, and functional benefit as a result of transplantation, likely as a result of growth factor secretion. Currently, we are looking at the effect of hESC-MNP transplants on the maturation of the pre- and post-synaptic AChR regions at the neuromuscular junction (NMJ), the earliest detectible pathology in Δ7SMA mice. Our laboratory is also developing a means of drawing transplanted motor neuron axons to peripheral muscles, to enhance repair. hESC-MNPs represent a biological tool to investigate human motor neuron development, and provide a clinically relevant cell population for the development of therapies to treat injuries or diseases characterized by motor neuron loss.

P-43 Clinical Trial Design For Phase I/IIA Embryonic Stem Cell Derived Motor Neuron Progenitor Transplant (MOTORGRAFT^{TM}) For Treatment Of Infantile

Spinal Muscular Atrophy Type I

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Spinal muscular atrophy (SMA) Type I is a terminal pediatric genetic disorder characterized by progressive motor neuron death due to mutations in the gene encoding the motor neuron survival protein SMN. This condition is lethal in infants and young children. MotorGraft™ is a high purity late stage motor neuron progenitor (MNPs) population derived from human embryonic stem cells and intended for use as a cell replacement therapy. Cell replacement treatment is expected to counteract SMA disease processes by two mechanisms: 1) replacement of dying motor neurons and 2) secretion by the transplanted cells of motor neuron specific growth factors for rescue of the host motor neurons.

The primary objective of the clinical trial is to evaluate the safety and tolerability of MotorGraft™ transplantation into the ventral horn of the spinal cord at multiple sites along the cranio-caudal axis in symptomatic SMA Type I infants. The secondary objective is to evaluate the ability of MotorGraft™ to improve function of damaged motor neurons.

There are many challenges in designing a clinical trial for a novel therapeutic approach in a terminal pediatric population. They include issues such as validated efficacy outcomes, survival time of terminal pediatric patients, selection of appropriate controls, inclusion and exclusion criteria selection, appropriate surgical approach, cohort design and importance of autopsy data.

In order to address the aforementioned challenges, multiple focus groups were held with experts in pediatrics, neurology, transplant surgery, neurosurgery and motor neuron disease research. The result of these meetings was a fully developed clinical program and trial design for MotorGraft™ transplantation in infants with SMA Type I. Key safety outcomes include evaluation for tumor formation and migration of cells in spinal tract. Exploratory efficacy outcomes include time to 16 hours of assisted ventilation, and frequency of respiratory infections requiring hospitalization.

P-44 Tumorigenicity, Allodynia, Biodistribution And Toxicity Study Of Human Embryonic Stem Cell (HESC) Derived-Motor Neuron Progenitors Following Transplantation Into The Spinal Cord In NOD/SCID Mice

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Type I infantile spinal muscular atrophy (SMA) is a pediatric genetic disorder characterized by progressive motor neuron death due to mutations in the gene encoding the motor neuron survival protein SMN. This deficit leads to total paralysis and death. We have developed methodologies for scaled manufacture of high purity motor neuron progenitors (MNPs) from human embryonic stem cells (hESCs) with the intent to develop a combination cell replacement and neuroprotective therapy for SMA. In this six-month study, we evaluated tumorigenicity, allodynia, biodistribution, and toxicity following transplantation of hESC-derived MNPs in the

spinal cord of NOD/SCID mice. In addition, we examined the threshold for undifferentiated hESC contaminants added to the hESC-derived MNP population that may lead to tumor formation. At multiple time points our analyses indicated that hESC-derived MNP transplantation did not result in tumors, allodynia, distribution of cells to other organs, or toxicity. Additionally, hESC-derived MNPs contaminated with 6% and 20% undifferentiated hESCs did not form tumors, while 100% undifferentiated hESCs did result in tumor formation. These data from a comprehensive safety study support the use of hESC-derived MNPs for the treatment of SMA.

P-45 Regenerated Corticospinal Tract Axons In Mice Form Synaptic Contacts In Segments Caudal To The Lesion

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We recently showed that normal C 57Bl/6 mice exhibit a limited regenerative growth of corticospinal tract (CST) axons after dorsal hemisection injury in which axons extend past the lesion via the ventral column and form elaborate terminal arbors in the gray matter caudal to the injury (Steward *et al*. J Neurosci. 28(27):6836-6847, 2008). The terminal arbors caudal to the injury have multiple boutons suggestive of synaptic contacts. Here we assess whether these boutons are in fact synapses. To address this question, we injected biotinylated dextran amine (BDA) into the right motor cortices of C57Bl/6 mice 45 days after the mice had received dorsal hemisection lesions. Blocks containing the lesion site were sectioned sagittally on a vibratome, stained for BDA, and embedded in Spurr's resin and sandwiched between layers of Aclar film to allow assessment by light microscopy. Stained CST axon arbors caudal to the injury were identified at the light microscopic level and individual boutons (possible presynaptic terminals) were identified. The blocks were then prepared for electron microscopy, and boutons identified at the light microscopic level were located. Of 7 boutons recovered so far, 5 are definitive synaptic contacts in that they appose postsynaptic membrane specializations. These results indicate that axons involved in this form of regeneration do form synapses on neurons in the gray matter caudal to the injury. In addition, there is a high probability that boutons identified at the light microscopic level as possible presynaptic terminals are in fact bona fide synapses that contact postsynaptic membrane specializations. The formation of synaptic contacts by CST axons that extend past a dorsal hemisection lesion creates a circuit that could contribute to recovery of motor function in segments caudal to the injury.

P-46 Autoimmunity After Spinal Cord Injury: ANTI-CRMP2 Antibodies Facilitate Regeneration In Vitro But Cause Pathology In Vivo

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Traumatic Spinal cord injury (SCI) causes glial activation and recruitment of innate and adaptive immune cells. Previously we showed that injury induces B lymphocyte proliferation in spleen and spinal cord, generating autoantibodies against multiple CNS antigens including glutamate receptors and nuclear antigens. Probing mouse CNS proteins with IgG isolated after SCI revealed over fifty possible auto-antigen targets. One of these targets was collapsin response mediator protein 2 (CRMP2), a cytoplasmic protein with punctuate membrane distribution that is important in axonal guidance, axon/dendrite polarity, and neural regeneration. CRMP2 is expressed in growing neurons, oligodendrocytes, and T-lymphocytes activated after CNS infection. To investigate the functional effects of autoantibody responses against CRMP2, we performed stereotactic microinjections of polyclonal anti-CRMP2 or control antibodies into naïve spinal cord gray matter. Also, anti-CRMP2 was co-injected with lipopolysaccharide (LPS), a potent microglial/macrophage activator, to access the effects of anti-CRMP2 antibodies in a site of inflammation. After seven days of observation, spinal columns were fixed, dissected, blocked, mounted onto slides, and stained for analysis. In parallel, in vitro dorsal root ganglion (DRG) neurons were incubated in anti-CRMP2 or control antibody, and growth was accessed using a semi-automated Scholl ring analysis. After microinjection, anti-CRMP2 generated significantly more inflammation and subsequent axon degeneration compared to rabbit serum or LPS alone. However, anti-CRMP2 with LPS had consistently smaller lesions than rabbit serum coinjected with LPS or LPS alone. In DRG culture, anti-CRMP2 treated neurons had significantly increased regeneration compared with rabbit serum treated neurons, indicated by the increased mean ring intersections. These results show that in a controlled setting, anti-CRMP2 can interfere with the regulation of axon growth, favoring regeneration. However, in an intact spinal column, anti-CRMP2 causes inflammatory pathology, suggesting that an antibody response could exacerbate lesion expansion after SCI.

P-47 Analysis Of Immune-Mediated Axon Injury In An In Vitro Microfluidic Chamber Model Of Multiple Sclerosis

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A major pathological hallmark of multiple sclerosis (MS) is inflammatory demyelinated lesions found in the central nervous system. Demyelination is not, however, sufficient to induce the functional deficits associated with MS. Our in vivo data suggest that axon injury and subsequent functional deficits are linked to recognition of the naked axon by CD8+ T cells, but the events leading up to CD8+ attack are unclear. To characterize the nature of axonal stress associated with demyelination and how this stress might contribute to the axon injury associated with MS, we have developed an in vitro microfluidic culture model of demyelination and axon injury. Cortical neurons were plated in microfluidic chambers to separate the axon tip from the cell body. A trophic factor conditioning cocktail was applied to the axons for 7 to 10 days, and then partially withdrawn overnight. Under these conditions, the neurons do not die; rather, the axons begin to display evidence of stress, including changes in neurofilament phosphorylation status. Spinal cord-

infiltrating leukocytes (SCILs) isolated from chronically demyelinated mice experiencing ongoing axon injury were added to the axon chamber. Live-cell imaging showed polarization of SCILs, as well as wash out-resistant interaction with axons and possible activity-induced lymphocyte cell death. Further, we found that the population of SCILs that does not attach to axons and is washed out of the chamber is depleted of CD45hi cells. We propose that demyelination leads to loss of axonal trophic support, that loss of trophic support leads to decreased neurofilament phosphorylation and impaired retrograde axonal transport, and that the resulting axonal stress predisposes axons to be recognized by SCILs. Our in vitro model provides us with a platform upon which to investigate the role of these events in axon injury associated with MS.

P-48 Deficient Fractalkine Receptor (CX3CR1) Signaling Confers Neuroprotection And Promotes Functional Recovery After Traumatic Spinal Cord Injury

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Fractalkine (FKN) is a key regulator of microglial and monocyte/macrophage function. In the healthy CNS, FKN is constitutively expressed by neurons while its receptor (CX3CR1) is expressed on microglia, suggesting a basal commuicating role for CX3CR1 signaling. CX3CR1 signaling also regulates monocyte infiltration into injured or diseased tissue. The extent to which CX3CR1 signaling regulates microglial and monocyte/macrophage function in the context of traumatic spinal cord injury (SCI) is unknown. Here we show that abolishing CX3CR1 signaling in SCI mice is neuroprotective and is associated with improved functional recovery but without marked changes in the magnitude of leukocyte infiltration at the site of injury. Instead, CX3CR1 deletion alters the phenotype of responding microglia/macrophages. Indeed, the number of $Ly6C^{low}$ macrophages was greatly reduced in CX3CR1-deficient ($CX3CR1^{GFP/GFP}$) mice. This corresponded to decreased expression of IL-6 and iNOS, suggesting that impaired CX3CR1 signaling inhibits the development of a pro-inflammatory microenvironment at the site of injury. Interestingly, arginase I levels, a marker for so-called alternatively activated or wound-healing macrophages, were not affected by CX3CR1 deletion. Our findings in SCI were supported *in vitro*. The oxidative capacity of cultured $CX3CR1^{GFP/GFP}$ microglia and macrophages was reduced in response to LPS, a canonical inflammatory stimulus. This effect was recapitulated in wild-type microglia/macrophages when stimulated in the presence of CX3CR1 blocking antibodies. Similarly, serum, a pro-inflammatory stimulus of relevance to SCI and other disorders associated with blood-brain barrier damage, failed to elicit a pro-inflammatory cytokine signature in primary microglia from CX3CR1-deficient mice. Thus, blocking CX3CR1 signaling may be a viable therapy for CNS disorders in which vascular injury occurs (e.g. stroke, SCI, traumatic brain injury).

P-49 Supraspinal Sensory Perception In Below-Level Allodynia After Moderate Spinal Cord Contusion In Rats

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A common, debilitating consequence of spinal cord injury (SCI) is neuropathic pain below the injury. Allodynia—sharp, intense pain to normally innocuous stimuli—is refractory to treatment likely because brain mechanisms of allodynia remain unidentified. Evidence of spared spinothalamic axons in SCI-induced allodynia contradict reports that insensate rats with SCI lack cortical activation using functional magnetic resonance imaging (fMRI). The purpose of this study was to confirm supraspinal sensory perception after moderate SCI and delineate the role of primary afferents and nociceptive axons in allodynia using fMRI and somatosensory-evoked potentials (SSEP). Adult rats assigned to Naïve (n=3) or moderate T8 SCI (n=16) groups had von Frey Hair and BBB testing through 35 days post SCI. SSEP recorded over S1 cortex after 100 single pulse stimuli (0.5 ms duration, 0.5Hz) to the sciatic nerve occurred 5 wks post SCI. fMRI was conducted using echo planar imaging in a 4.7T/40cm Bruker MRI System with a 400 mT/M gradient insert. Functional scans of pinprick stimulation to the hindpaw consisted of 11 total segments: 6 "off" segments (30 sec) alternating with 5 "on" segments (15 sec). Voxel number and maximum Z score in region-of-interest analysis for the contralateral S1 cortex and thalamus were conducted with FSL software. Both fMRI and SSEP confirmed cortical activation to below-level stimulation after SCI, although the intensity of fMRI activation (voxels and Z scores) decreased in S1 cortex and thalamus after SCI. SSEPs had 4 positive peaks (P1-4) temporally corresponding with 4 sensory axon types (1a, Aβ, Aδ, c). The P1 and P4 peaks showed pain-related delays in latency or onset/offset. SCI-induced hypersensitivity negatively correlated to longer P1 and P4 latencies. Our findings indicate that sensory perception rather than numbness exists after SCI and SSEPs have good utility in differentiating below-level allodynia with the potential to be an accurate biomarker of SCI-induced neuropathic pain.

P-50 Repairing Spinal Cord Circuitry After Injury Utilizing Neural Stem Cells

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The adult spinal cord does not support endogenous neurogenesis, presenting a challenge for therapeutic strategies aimed at neuronal cell replacement after spinal cord injury (SCI). Previous studies have shown that multipotent neural stem cells (NSC) transplanted into SCI survive poorly and differentiate mostly into glial cells. Therefore, it is necessary to promote survival of transplanted NSC while also providing cues for neuronal fate restriction. We tested the concept of neuronal cell

replacement using pre-differentiated NSC to replace ventral interneurons in a gray matter injury model aimed at disrupting the central pattern generator (CPG) in the lumbar spinal cord. To generate an injury in which only a portion of a neuronal circuit is ablated, we used ibotenic acid (IBO) injection, creating a focal ventral gray matter lesion. Injection was directed to one spinal segment so that only part of the CPG circuitry was damaged. Fate-restricted precursor cells were generated through pre-differentiation of multipotent NSC using conditions that recapitulate the microenvironment of the developing ventral spinal cord. We optimized protocols developed for embryonic stem cells by treating NSC prepared from embryonic rat spinal cord with growth factors and varying concentrations of retinoic acid (RA) and a sonic hedgehog agonist. The effects of these morphogens at different time points were evaluated by immunocytochemistry and real-time PCR using markers for multipotent NSC, neuronal precursors and specific neuronal phenotypes. Within one week we observed cells with neuronal morphology and expression of transcription factors specific to motoneuron- and interneuron-precursors. We transplanted these cells into the IBO-induced injury and found that they survived and maintained their neuronal identity. We are currently investigating the extent of graft integration with host tissue. In future experiments we will study the physiological and behavioral deficits associated with the injury and the extent of circuitry repair promoted by this strategy.

P-51 Promoting Connectivity Of Neural Stem Cells With The Injured Spinal Cord

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Spinal cord injury (SCI) results in loss of neurons and disruption of long axonal tracts. We are studying the potential of neural precursor cells (NPC) to replace neurons at the site of the lesion and to reconnect long tracts with appropriate targets via a neuronal relay. In order to restore neuronal connections NPC must produce mature neurons in the SCI microenvironment, and those neurons must integrate with host tissue by extending axons out of the graft towards an appropriate synaptic target. We have previously shown that grafting a mixed population of neuronal and glial restricted precursors (NRP and GRP, respectively, prepared from AP transgenic rats) in the injured spinal cord produces neurons with glutamatergic and GABAergic phenotypes. When NRP/GPR are grafted in a dorsal column injury model and presented with a gradient of BDNF rostral to the lesion site, NRP-derived axons are guided through the white matter by the neurotrophin gradient. In the current study we examine the ability of NRP to form postsynaptic connections with regenerating host axons at the graft site and presynaptic connections with target neurons in the dorsal column nucleus (DCN). To study postsynaptic integration we grafted NRP/GRP with permissive matrices, traced ascending sensory pathways and found that host sensory axons can regenerate into NRP/GRP grafts and express synaptic markers. To study presynaptic integration, we used neurotrophin gradients to guide axons from NRP/GRP grafted into a C1 dorsal column injury into the target, the DCN. We examined graft-derived axons for evidence of synaptic connections. The analysis will include a combination of immuno-EM examining AP-positive graft axons and tract tracing from the thalamus. Our findings provide the proof of concept that NPC grafts

can participate in replacement of local neuron populations and reconnect long distance axon tracts.

P-52 Equivalence Of Conventionally-Derived And Parthenote-Derived Human Embryonic Stem Cells

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As human embryonic stem cell (hESC) lines can be derived via multiple means, it is important to determine particular characteristics of individual lines which may dictate the applications to which they are best suited. The objective of this work was to determine whether parthenote-derived and conventionally-derived hESC lines are equivalent in the undifferentiated state and during neural differentiation. Two conventionally-derived lines and two parthenote-derived lines were exposed to the same expansion conditions and subsequent differentiation protocols. Growth rates and gross morphology were recorded during expansion. Triplicate PCR arrays were carried out to determine expression of developmentally relevant genes. Global DNA methylation analyses was carried out to compare epigenetic profiles. Lines were exposed to two differentiation protocols to determine differentiation potential within the neural lineage. PCR arrays on undifferentiated samples were not sensitive enough to detect any major differences in gene expression between the lines, however, PCR arrays specific for extracellular matrix genes on early neural progenitors revealed differences between parthenote and conventional lines. Methylation microarrays, when filtered to include only imprinted genes, also showed differences between line types. Parthenote lines exhibited impaired proliferation in undifferentiated conditions. After differentiation protocols, parthenote lines yielded lower quantities of cellular product and exhibited impaired maturation, consistent with differentially methylated imprinted genes of developmental significance. These data demonstrate that parthenote-derived lines have reduced differentiation potential compared to standard hESC and that molecular differences were most pronounced in imprinted genes.

P-53 Role Of *EMX1* In Neural Stem Cell Renewal And Regeneration

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Corpus callosum as the principal fiber tract connecting the left and right hemispheres plays an important role in mediating functional recovery following unilateral brain injury. Our previous studies showed that the deletion of homeobox *Emx1* gene not only led to a disruption of corpus callosum but also reduced hippocampal neurogenesis in sham and mice with experimental focal stroke. A proteomic comparison using 2-D gel electrophoresis and tandem mass spectrometry

between the wild type and *Emx1* null mice revealed a distinct pattern of expression in F-actin depolymerization factor cofilin, which is an important regulator of cell migration and cell cycle progression during cortical development. The aim of this study is to determine whether the reduced regeneration in the *Emx1* mutants originates from defects in neurogenesis during early stage of brain development. To determine the effect of *Emx1* gene deletion on neural stem cell (NSC) pool, self-renewal and NSC migration, neurosphere assay and migration assay were performed with E14 embryonic brains from *Emx1* null (KO) and wild type (Wt) mice. Our data suggest that Emx1 deletion reduced the frequency and secondary renewal of NSCs but did not affect neuronal or glial differentiation. Transwell migration assays showed a tendency of reduced migratory capacity of *Emx1* KO NSCs in response to vascular endothelial growth factor (VEGF) and serum. In summary, the defects observed in the neurosphere frequency, self-renewal and migration capacity of NSCs during the embryonic stage of KO *Emx1* mice might contribute to the reduced neurogenesis in the adult hippocampus of sham and stroke mice. Ongoing investigation will determine whether *Emx1* deletion affects the cofilin pathway that might underlie the defects in VEGF-induced NSCs migration and reduced regeneration following experimental stroke.

P-54 Sex, Estrogen, And Recovery After Spinal Cord Injury

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INTRODUCTION: Behavioural recovery following spinal cord injury (SCI) is associated with neuroanatomic plasticity. Estrogens are involved in neuroanatomic plasticity. We hypothesized that the sex of an animal is an important factor to consider when examining sensorimotor behavioural recovery following SCI and that blockade of estrogen receptors would impair behavioural recovery. It is thought that estrogen influences the serotonergic (5-HT) system via neurons in the dorsal raphe nuclei that express estrogen receptors. Since 5-HT plays a key role in locomotor circuitry and has been shown to alter locomotor recovery after SCI, 5-HT was used as an indicator of neuroanatomic plasticity. **METHODS:** We examined sensorimotor behavioural recovery in 1) male rats treated with vehicle, 2) female rats treated with vehicle, and 3) female rats treated with the estrogen receptor antagonist, ICI 182,780 (ICI) before and after C3 left lateral spinal cord hemisection. Endpoint measures (tapered beam, ladder rung-walking, paw preference, inclined plane, BBB locomotor rating scale, BBB-subscore rating scale) were collected before and at weeks 1, 2, 4, 6, and 8 after SCI. Ground reaction forces and kinematic data were collected before SCI and at 8 weeks post-SCI. Serotonergic immunoreactivity was examined at C8 and L5, in lamina VIII of the gray matter. **RESULTS:** On several of the behavioural tasks, ICI-treated and male animals performed poorly compared to non-ICI female animals. Statistically significant differences were most pronounced at 4 weeks following SCI, though some differences persisted at 8 weeks after SCI. Non-ICI-treated animals had greater 5-HT immunoreactivity compared to ICI-treated females and male animals treated with vehicle alone. **CONCLUSIONS:** Estrogen receptors are involved in behavioural recovery following SCI. We provide evidence that estrogen is likely acting to improve recovery, in part, through neuroanatomic plasticity of descending 5-

HT fibers. Estrogen or selective estrogen receptor modulators (SERMs) may prove valuable as therapeutants following SCI. **SUPPORT:** NSERC grant to AAW; Queen Elizabeth II Scholarship to SCKN.

P-55 Impaired Local Vasodilation May Contribute To Cardiovascular Dysfunction Following Spinal Cord Injury

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Disordered cardiovascular control following spinal cord injury (SCI) is both dangerous and detrimental to quality of life. This is particularly true for individuals with high SCI, many of whom experience both autonomic dysreflexia (AD) and orthostatic hypotension, and thus experience pronounced fluctuations in blood pressure. We do not know how this blood pressure lability affects the resistance vessels, or cardiovascular function over the long-term.

Mechanisms underlying AD remain controversial; available data suggest that injury-induced plasticity is involved, and that the changes responsible occur both in the nervous system and in target organs, including the resistance vessels. These experiments investigate the potential for impaired local vasodilation in resistance vessels below the level of SCI to contribute to the pronounced hypertension observed in episodes of AD. Complete spinal cord (T3) transection was performed in male Wistar rats; sham-injury involved laminectomy and durotomy. Animals survived for one or four week(s). On the last day of the experiment, AD was examined by recording arterial pressure and heart rate during balloon distension of the colon. Segments of mesenteric artery were harvested and mounted in a four-chamber myograph for characterization of their vasoactive properties.

Mesenteric arteries below SCI were hypersensitive to phenylephrine (PE), which mimics norepinephrine, the primary neurotransmitter effecting vasoconstriction. To examine mechanisms underlying SCI-induced PE sensitivity, we investigated endothelial-mediated vasodilation. Arterial segments from animals with SCI exhibited normal vasodilatory responses to acetylcholine, and PE-sensitivity was not attenuated by L-NAME; therefore, nitric oxide-induced vasodilation remained functional caudal to SCI. However, PE-sensitivity was attenuated in the presence of indomethacin and NS-398 (a COX-2 inhibitor). These findings suggest that COX-2, an enzyme associated with many types of pathological inflammation, may also contribute to cardiovascular dysfunction following SCI.

P-56 Combined Cellular & Dibutyryl Cyclic AMP Delivery To The Transected Spinal Cord Via Oligo [Polyethylene Glycol) Fumarate] Hydrogels

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Re-establishment of functional connections after SCI requires injured axons to grow through the graft, enter normal tissue, find target cells, and establish synapses to complete a functional circuit. This study describes the use of oligo [(polyethylene glycol) fumarate] (OPF) hydrogel scaffolds as vehicles to deliver sustained dibutyryl cyclic adenosine monophosphate (dbcAMP) to the transected spinal cord. cAMP mediates diverse functions within the nervous system, including cell survival, axon guidance and neurite outgrowth. dbcAMP release was assessed from OPF hydrogels in which the dbcAMP was encapsulated in polylactic-coglycolic acid (PLGA) microspheres. Functionality of the released dbcAMP was assessed using neurite outgrowth assays in PC12 cells. Delivery of dbcAMP to the transected spinal cord was accomplished through incorporation of the microspheres within OPF seven channel scaffolds, equal in diameter to the spinal cord. These channels were loaded with schwann cells or mesenchymal stem cells in a matrigel substrate. Our results showed that encapsulation of dbcAMP in microspheres lead to prolonged release over one month. Functionality of the dbcAMP was unaffected by this encapsulation process. These microspheres were then successfully incorporated into scaffolds and implanted in the transected thoracic spinal cord, where they remained for one month. Our findings demonstrate the feasibility of incorporating PLGA microsphere technology and cellular transplantation for spinal cord transection studies. It represents a novel sustained delivery mechanism within the transected spinal cord and provides a platform for potential delivery of other therapeutic agents.

P-57 Promoting Functional Recovery After Spinal Cord Injury By A Small-Molecule P75 Ligand That Disrupts Prongf Binding To P75 And Crsses Blood-Brain-Barrier

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In previous studies, we demonstrated that small molecule, non-peptide p75 ligands that mimic the loop1 domain of NGF and NT3 block proNGF binding to p75 in cultured oligodendrocytes (Massa et al., JN, 2006). A number of p75 ligands have been identified that cross the blood-brain-barrier following oral administration. The goal of this project was to determine whether a prototype p75 ligand can attenuate oligodendrocyte death during Wallerian degeneration after spinal cord injury, thereby promoting overall functional recovery when it is delivered long after initial impact. A spinal cord contusion model was used in mice using a 50 Kdyne drop weight force at T9 using Infinite Horizons Impactor. Following injuries, the p75 ligand was delivered at 10, 25, 100, and 320 mg/kg using oral gavage beginning 4hr after injury for a period of 42 days. Control injured mice received the vehicle and laminectomized control mice received the same treatments in parallel. The ligand exhibited no toxicity based on survival analyses and the rate of weight loss. Since NGF plays a critical role in nociception, we have also assessed whether the p75 ligands modulate overall pain sensation after the injury. The ligand treated mice responded similarly to the vehicle-treated control in mechanical allodynia and thermal hyperalgesia tests, suggesting that the ligands do not potentiate the pain sensation after the injury. We are continuing to investigate motor coordination with swimming, gridwalk, and BMS behavioral assays, and plan to present the final analyses at the meeting. These behavioral studies will also be complimented with cell biological analyses to

determine whether the p75 ligands promote oligodendrocyte survival and reduce the extent of myelin loss.

P-58 Spontaneous Afferent Recovery In Spinal Segments Afffected By Spinal Cord Injury: Evoked Potential Findings And Implications For Clinical Trials

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Segmental dermatomal evoked potentials (EPs) provide comparable neurophysiological readouts to conventional mixed nerve EPs, but can also yield information regarding individual spinal segments near the level of spinal cord injury (SCI). This study aimed to determine the psychometric properties of dermatomal contact heat (CHEPs) and somatosensory evoked potentials (dSSEPs) to: 1) monitor the stability of chronic SCI (>1 year), and 2) track changes during spontaneous recovery from SCI.

Initial and follow-up dSSEPs and CHEPs were recorded from cervical and thoracic dermatomes of individuals with tetra- and paraplegia. Unaffected dSSEPs and CHEPs were recorded with preserved electrical and contact heat perception thresholds, EPT and HPT respectively, at and above the level of SCI. During recovery, initial dSSEPs unaffected by SCI revealed no significant change in N1 latency and EPT on follow-up ($p>0.05$). In comparison, the N1 latency of dSSEPs initially delayed by SCI at and below the level of lesion significantly decreased on follow-up ($\Delta=-3.1\pm2.9$ms, $p<0.01$), but without a corresponding increase in sensitivity of EPT ($p>0.05$). The conversion of abolished-to-recordable dSSEPs was also observed, and often preceded by preserved initial EPT and a concomitant recovery of EPT at follow-up. Repeat examination of dSSEPs and CHEPs in individuals with chronic SCI revealed strong intraclass correlation coefficients for the interpretation of latency and amplitude in unaffected and delayed recordings (>0.70, $p<0.01$), and no significant change in the interpretation of abolished recordings.

Segmental EPs can be reliably recorded from cervical and thoracic dermatomes to monitor afferent stability in a spinal segment chronically affected by SCI, and track changes in spontaneously recovering spinal segments. The findings indicate that segmental EPs may be useful to measure safety of locally applied therapeutics in early phase clinical trials, as well as document the efficacy of interventions where the standard neurological assessment might not detect subtle therapeutic effects.

P-59 Cyclic Amp Mediated Serotonergic Axon Growth And Functional Recovery After SCI: Continuous Versus Transient Elevation

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Injury to the spinal cord produces significant anatomically discontinuity and functional loss below the level of the lesion. In a system capable of only limited endogenous repair, exogenous manipulations are required to promote meaningful axonal re-growth and functional restitution. We have previously demonstrated that a combination of Schwann cell (SC) implantation and elevation of the second messenger, cyclic AMP (by both analog supplementation and the inhibition of its hydrolysis) can promote significant serotonergic axon growth and functional recovery after SCI (Pearse et al., 2004). In the present work we investigated whether cyclic AMP analog delivery to the spinal cord alone, in SC implanted, thoracically contused adult rats, either as a single (intraspinal injection at four locations, two rostral and caudal to the SC implant) or continuous (intrathecal catheter delivery at the caudal edge of the implant for 2 weeks) administration could enhance cylic AMP levels in the brainstem and motor cortex, facilitate serotonergic growth beyond the SC implant to the L2 lumbar cord and improve functional recovery in a more severe contusive spinal cord injury (SCI; 25.0 vs. 12.5 mm weight drop; MASCIS impactor). For these investigations cyclic AMP delivery was commenced at one week post-SCI, at the time of SC implantation, and histological and functional outcomes were assessed from three days to four weeks post-injury. We found that while continuous cyclic AMP analog delivery to the spinal cord led to significant increases in supraspinal cyclic AMP levels, serotonergic axon growth beyond the SC implant and improved open-field locomotion over SC implanted, SCI controls, a single administration of the cyclic AMP analog was without effect on all the parameters assessed. The current work demonstrates in this paradigm that sustained cyclic AMP delivery (elevation) at the level of the spinal cord is required for axonal re-growth and functional recovery after SCI. Support: NIH NINDS R01 NS 05628-01.

P-60 DELTA/NOTCH-LIKE, EGF-Related ReceptoR (DNER) Regulates Brain Lipid Binding Protein (BLBP) Expression In Olfactory Ensheathing Cells

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The success of continuous neurogenesis in the olfactory system is thought to be due, at least in part, to its unique glia – olfactory ensheathing cells (OECs). OECs bear characteristics of both peripheral and central glia. They serve to ensheath, guide and promote growth of olfactory receptor neurons (ORNs) throughout both development and adult life. Brain lipid binding protein (BLBP; FABP-7; B-FABP) is most highly expressed by radial glia during embryonic development. BLBP is largely down-regulated in the adult CNS, however BLBP expression is retained in the adult by special subpopulations of glia including OECs. BLBP expression is induced in radial glia via Notch signaling, but it is not known if these same mechanisms maintain BLBP in the adult CNS in radial glial-like cells. Delta/Notch-like EGF-related receptor (DNER), a transmembrane protein expressed by purkinje cells, has been implicated in the regulation of BLBP expression and morphology in Bergmann glia during cerebellum development. DNER signaling in this area acts through Notch1-Deltex-dependent non-canonical signaling. We have found that DNER is expressed in more mature ORNs, adjacent to BLBP expressing OECs. Immunofluorescence shows that this close relationship between BLBP expressing cells and DNER

expressing cells also appears to be retained in specialized areas such as the hippocampus and spinal cord, throughout mouse development. OECs lose BLBP expression over time in vitro and in vivo when ORNs are removed by bulbectomy; this effect appears to be recapitulated when DNER is knocked out in vivo. Expression of BLBP in vitro increases when OECs are co-cultured with DNER. This work suggests that DNER expressed in neurons may regulate BLBP expression in neighboring glia. The activation of this pathway may increase the inherent plasticity seen in OECs responsible for their ability to support neuronal survival and outgrowth in vivo and in vitro.

P-61 Loss Of Netrin-1 In The Hematopoietic System Modulates Macrophage Infiltration, Increases Lesion Volume And Reduces Locomotor Recovery After Spinal Cord Contusion

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Spinal cord (SC) injury often results in disruption of the blood brain barrier, allowing cells from the immune system to infiltrate the SC. We previously showed that adult spinal cord progenitor cells (aSCP) are highly sensitive to the repulsive cue, netrin-1 (Ntn-1). Ntn-1 is constitutively expressed at low levels in the adult SC and is increased at the injury site 4 days post-injury (DPI), remaining elevated for at least 10 days. It was previously demonstrated that infiltrating macrophages express Ntn-1 within the zone that eventually becomes the lesion core. We hypothesized that aSCP are directed away from the injury core by a repulsive force driven by the invading immune system. We predicted that down-regulation of Ntn-1 expression at the injury site would lead to an increased number of aSCP at the lesion epicenter, thereby increasing tissue remodeling and improving locomotor recovery. We created a chimeric mouse by transplanting Ntn-1 deleted fetal liver cells into a wild-type, lethally irradiated mouse that lacks the capacity to produce Ntn-1 by peripheral macrophages. We evaluated motor function by the open-field Basso Mouse Scale to correlate aSCP remodelling of the lesion core with recovery of function. Surprisingly, at 45 DPI mice with the Ntn-1 -/- hematopoietic stem cells (HSC) displayed worse locomotor recovery than control mice expressing Ntn-1. Analysis of SC tissue clearly showed a much larger injury volume and significantly greater infiltration of macrophages at the injury core in HSC Ntn-1 -/-. We found that reactive macrophages are slower to infiltrate and slower to withdraw from the injury site in the absence of HSC Ntn-1 expression. Deleted Ntn-1 in the hematopoietic system seems to affect the migration of macrophages, creating a delayed but exacerbated macrophage infiltration, still intense at 45 DPI.

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P-62 Improved Functional Recovery Using Human Bone Marrow Stromal Stem Cells (hBMSCs) From Spinal Cord Injured (SCI) Patients Transplanted Into The Acute And Chronic Injured Rat Spinal Cord

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Multipotent hBMSCs from SCI patients were used to stimulate sparing and regeneration of descending neural pathways following acute and chronic moderate contusive SCI. Retrovirally transduced GFP-positive hBMSCs ($hBMSC^{GFP}$) were transplanted into immunologically deficient (Nude) rats subjected to moderate SCI (10g/12.5mm/T10) using an NYU impactor device. The therapeutic potential of $hBMSC^{GFP}$ was assessed both behaviourally and anatomically using immunohistochemistry. In both acute and chronic SCI studies, animals transplanted with hBMSCs consistently showed a switch from non-coordinated locomotor function to marked, coordinated locomotor recovery at around 4wks post $hBMSC^{GFP}$ transplantation (open field BBB and computer generated functional (Catwalk) analyses), and had more intact spinal tissue (sparing) than controls. Axonal and glial specific markers were observed on immunostained spinal cord sections in close proximity to donor $hBMSC^{GFP}$s within the lesion site at 1-2wks after transplantation, with no evidence of $hBMSC^{GFP}$ transdifferentiation and the absence of grafted cells was noted 8wks after transplantation using a human nuclear antigen and GFP markers. This coincided with a large increase in $ED1^{+}$ macrophages within the lesion and both rostral and caudal spinal cord tissue. Despite the dramatic improved functional recovery by approximately 3wks after $hBMSC^{GFP}$ transplantation (acute and chronic), extensive analysis of retrograde (fluorogold) labelled pathways revealed no new regeneration. This suggests that hBMSC therapy can successfully be incorporated into strategies developed to alter the acute and chronic injured host microenvironment and promote tissue sparing/repair of the mammalian spinal cord.

P-63 Human Bone Marrow Stromal Stem Cells (hBMSCs) From Spinal Cord Injured (SCI) Patients Transplanted Into Rat Spinal Cords After Complete-Transection

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Bone marrow stromal cells (BMSCs) have emerged as a promising candidate for treatment of spinal cord injury (SCI) in a number of animal models. However, the mechanism(s) responsible remains undefined due to disadvantages associated with incomplete transection models employed. Our work with human BMSCs (hBMSCs) from SCI patients shows dramatically improved anatomical and functional (behavioural) recovery after acute and chronic contusive (incomplete) SCI in rats, and suggests that hBMSCs may promote endogenous host responses that result in improved functional recovery and enhanced tissue sparing after SCI. This clinically

relevant and promising therapeutic strategy was extended to complete transection SCI to provide indisputable evidence of true axonal regeneration, the endogenous host stem cell mechanism (including proliferation/growth promotion), and interactions with host glial, astrocyte and oligodendrocyte populations. HBMSCs and rat BMSCs (rBMSCs) transplanted directly into the lesion sites of acute (1wk) and chronic (3mo) complete-transected spinal cords were analysed. Human and rat dermal fibroblasts (hDFs and rDFs) were used as control cells. The use of the scar reducing compound Decorin to counteract the inhibitory environment of the lesion site via its ability to suppress inflammation, CSPG expression and astroglial scar formation as well as providing a matrix to facilitate repopulation of the injury and regeneration was also investigated. Preliminary data reveals that functional recovery (assessed using several tests, including our powerful Catwalk automated gait analysis) in all donor cell treatment groups reached BBB scores of 8 (sweeping with no weight support) after 6wks. Morphologically, GFAP, Pan-NF, and Beta-III-Tubulin expression profiles were present immediately caudal and rostral to the injury site in all treatments, as was laminin, CGRP, Cam 2, and 5HT. The efficacy of the hBMSC based cellular therapy with or without Decorin may provide an avenue for a combination therapy that further enhances the level of functional spinal cord repair.

P-64 Which MSC Should We Transplant? A Comparison Of Industrial High Content Screening Versus Published Methods To Assay The Effects Of Mesenchymal Stem Cells On Neurite Outgrowth *In Vitro*

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Bone marrow mesenchymal stem cells (MSC) promote axonal growth, neuronal survival and functional recovery in animal models of spinal cord injury (SCI) to varying levels. We have used high content screening, using the Cellomics Ltd. Arrayscan platform, to examine the effects of human MSC-conditioned medium (MSC-CM) on neurite outgrowth from the human neuroblastoma cell line SH-SY5Y and from explants of chick dorsal root ganglia (DRG). These analyses were compared to previously published methods, which involved hand-tracing and measuring individual neurites with Image J or IP Lab software. Both methods demonstrated that MSC-CM promoted neurite outgrowth. Each method showed (i) that the proportion of SH-SY5Y cells with neurites (≥10µ) increased from 6-8% in non-conditioned medium to ~20% in MSC-CM within 48 hours; and (ii) that the number of neurites/ differentiated SH-SY5Y cell was significantly increased in the presence of MSC-CM. For high content screening, the analyses were performed to completion within minutes, testing multiple samples of MSC-CM and in each case measuring ~250 000 SH-SY5Y cells. In contrast, the measurement of neurite outgrowth from ≥200 SH-SY5Y cells in a single sample of MSC-CM by hand-tracing took at least 1 hour. The high content analyses provided additional measures of increased neurite branching in MSC-CM (compared with control medium) within the same short time-frame. MSC-CM was also found to stimulate neurite outgrowth and branching in chick DRG explants using either method, although the number of outcome measures from DRG explants via the high content analysis was more limited than for the SH-SY5Y cell line. In conclusion, industrial high content analyses have provided a time-efficient means of testing the capacity of human MSC to stimulate neurite outgrowth *in vitro*.

The potential of such methodology may have application to the development of rapid pre-clinical screening techniques for optimized MSC transplantation for spinal cord injury.

P-65 Genetically Altered Schwann Cells Improve Histological And Functional Restoration Following Transplantation Into Acute And Chronic Models Of Spinal Cord Injury

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Spinal cord injury (SCI) results in neuronal degeneration and subsequent loss of associated motor and sensory functions. Schwann cells (SCs) transplantaion into the injured spinal cord are an attractive experimental therapeutic due to their intrinsic neuronal growth promoting properties and ability to guide and remyelinate the regenerated axons. However, they provide only a modest recovery of function after SCI due to their inability to outwardly migrate from the lesion site so as to sufficiently guide axons into and from the lesion site as well reach areas of demyelination. In the current study, we have genetically altered SCs to over-express polysialyl transferase (PST) prior to their transplantation following SCI to improve their migration capacity within the injured cord by altering their surface properties of SCs. Regulated expression of PST in SC increases surface production of polysialic acid (PSA) and reduce inter-cell adhesion allowing migration across the glial scar. Here, we have compared the ability of PSA-modified SCs in acute and chronic models of incomplete SCI, in the former case, the injury environment is easy to modulate but for chronic SCI, no effective treatment is available to date due to numerous intrinsic and extrinsic barriers to repair. Acute SCI animals revealed significant rostral-caudal migration (~3- 5 mm) of the PST-GFP-SCs from the implantation/lesion site compared to the control GFP-SCs, which remained confined to the site of injury and BDA-anterograde tracing from the motor cortex showed extensive corticospinal axon growth within and surrounding the implantation site. Behavioral analysis of PST-GFP-SC implanted acute animals revealed significant improvement in open-field locomotor performance and foot placement. Chronic animals showed some migration (~2mm) but significantly less compared to the acute animals and only modest improvement of locomotor function. PSA-expressing SCs transplanted post-SCI appear to support regeneration and functional restitution after both chronic and acute SCI. **Support:** NYS Department of Health and the Craig H. Neilsen Foundation

P-66 Skin-Derived Precursors Differentiated Into Schwann Cells Contribute To Repair After Transplant Into Chronically Injured Spinal Cord

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Cell transplantation has emerged as a popular candidate therapy for spinal cord injury because this approach has the potential to replace lost glial and neuronal cells, fill/bridge the lesion cavity, and promote axonal re-growth after spinal cord injury (SCI). However, the best candidate cell for a transplantation-based treatment of SCI remains a matter of intense debate. Our laboratory has previously shown that Schwann cells differentiated from skin-derived precursors (SKP-SCs), when transplanted 7 days after contusion injury, promote neuroprotection, histological repair, and functional recovery in rats. SKPs are potentially suitable for autologous transplantation but the therapeutic potential of SKP-SCs in the more clinically relevant chronic injury environment has not yet been investigated. Here, we transplanted one million cells into the lesion site of rats at five or at eight weeks post T9/T10 contusion injury. Behavioural assessments including the BBB, Ladder and Catwalk were used in the eight-week group to access functional locomotor improvements and are still underway at the time of abstract submission. Histological results from the five-week group indicate that the transplanted SKP-SCs group showed variable cell survival. Surviving SKP-SCs managed to bridge the lesion and integrate into the spared rim of the (GFAP positive) host spinal cord where many myelinated bone fide host axons. Furthermore, the average cavitation volume was significantly less in the SKP-SC group and tissue bridges were often filled with axons ensheathed by P0-positive (Schwann cell) myelin of endogenous and transplant origins. SKP-SC transplantation resulted in a significant increase in the presence of endogenous Schwann cells in the injured cord, but there was less astrocyte hypertrophy observed in areas containing the transplanted SKP-SCs. In conclusion, our initial 5-week delayed transplant indicated that the SKP-SCs are proficient at promoting repair, and we expect to present similar results from our 8-week group, which is currently being analysed.
This work was supported by grants from the Stem Cell Network (Canada) and the Canadian Institutes of Health Research (CIHR).

P-67 Schwann Cells Generated From Skin-Derived Precursors Show Discrete Advantages Over Those Harvested From Peripheral Nerve After Transplantation Into Incomplete Cervical Spinal Cord Injury

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We previously reported that Schwann cells generated from skin-derived precursors (SKP-SCs) facilitate axonal growth/sprouting into the lesion following delayed transplantation in the contused thoracic spinal cord of rats. More recently, we assessed SKP-SCs in an acute incomplete cervical injury (left C4/5 dorsolateral funiculus crush). Compared to media control, SKP-SCs enhanced branching of rubrospinal axons as well as recovery of forelimb function during vertical exploration (rearing/cylinder test). Here, we used the same injury model to compare acute transplants of neonatal rat SKP-SCs, peripheral nerve-derived SCs (PN-SCs), and fibroblasts. Both Schwann cell groups showed significant improvements in body weight distribution during locomotion (CatWalk analysis) compared to the fibroblast

group, with the SKP-SCs showing some improvements beyond those of PN-SCs. Red nucleus evoked electromyograms (EMGs) in the injured left forelimb showed that the efficacy of the rubrospinal tract in the SKP-SC group most closely resembled that of uninjured control animals, but average motor thresholds did not significantly differ among any of cell-treated groups. Both Schwann cell groups had significantly larger average graft volume than the fibroblast group. Schwann cells from both sources also behaved very similarly in vivo, partially bridging the lesion site and myelinating axons in and adjacent to the lesion. Importantly, compared to PN-SCs, the SKP-SCs elicited less reactive gliosis from the astrocytes surrounding the lesion site. This represents a potential advantage for SKP-SCs over PN-SCs, as less reactive gliosis is typically associated with enhanced axonal growth/sprouting. Taken together these data indicate that SKP-SCs show discrete advantages over PN-SCs after transplantation into the injured spinal cord. This adds further to the rationale to develop a SKP-SC treatment for spinal cord injury, as skin is easier to harvest and does not necessitate any risk of major nerve injury in providing a source of cells for autotransplantation. This work was supported by grants from the Stem Cell Network (Canada) and the Canadian Institutes of Health Research (CIHR).

P-68 Correlation Of GFAP Positive Processes With Regenerated Brainstem Axons In A Schwann Cell Bridge And BBB Scores Following Spinal Cord Transection Injury

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Complete transection of the thoracic spinal cord and implantation of a Schwann cell (SC) bridge is one of the most rigorous models to assess the regeneration of CNS axons. Previous work with this model found that implantation of a SC bridge alone results in the regeneration of intraspinal populations into the bridge. When a SC bridge is combined with additional treatments such as the application of growth factors, the elevation of intracellular cAMP, and/or the use of chondroitinase, supraspinal axons also regenerate into the bridge. Surprisingly, in our study of AAV-GFP-labeled brainstem axons, we observed up to ten percent of the axons to regenerate into a SC bridge and up to two percent to cross the entire bridge without additional treatments. Rostral spinal cord/SC bridge interfaces varied from sharp boundaries to irregular ones where GFAP positive processes entered the bridge. It was at the irregular boundaries that the percentage of brainstem axon regeneration correlated positively with the presence of GFAP positive processes entering the bridge. After six weeks, BBB scores also correlated with the total number of GFAP positive processes found at both the caudal and rostral interfaces; the BBB scores were dependent upon the presence of GFAP positive processes at both interfaces, in contrast to the brainstem axons. BBB scores (3.5-8) did not correlate, however, with the regeneration of brainstem axons, suggesting that following complete transection, the central pattern generator and/or the regeneration of intraspinal axons may be responsible for BBB scores up to 8. In sum, a SC bridge alone is permissive for some brainstem axon regeneration when interfaces exhibit GFAP positive processes crossing from the spinal cord into the bridge, and this should be examined when assessing treatment strategies designed to enhance supraspinal axon regeneration

and/or hind limb locomotion. (Supported by The Miami Project to Cure Paralysis and the Bouniconti Fund).

P-69 Comparison Of Cultured SCs And Freshly Isolated Pre-Degenerated Peripheral Nerve Following Transplantation Into The Contused Rat Spinal Cord

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Regeneration of CNS axons can occur in response to transplantation of peripheral nerves (PN), including intact predegenerated nerves (PDNs), dissociated PDNs, or their major cellular components, SCs and activated macrophages. Despite years of transplanting SCs into the spinal cord, many questions remain as to how best prepare the cells for transplantation clinically. Should the SCs be cultured and purified? Or should they be transplanted with minimal processing? SC yields are low when isolated from intact nerves but can be increased either by culturing SCs with growth factors or by crushing the nerve before cell collection. Both the latter can improve behavioral and histological outcomes following transplantation into the contused spinal cord. A direct comparison, however, has not been performed. Here we compared the efficacy of cultured SCs with freshly dissociated PDNs. Cells were transplanted into Fischer rats seven days after a moderate IH spinal cord contusion injury (206 +/- 1.1 KD). Motor (BBB, footprint, ladder and catwalk) and sensory (von Frey and Hargreaves) function over 11 weeks was measured. GFP+ SCs and NF+ axons were present within both graft types; SCs in PDN grafts were more dispersed and at a lower density compared to cultured SC transplants. Despite graft survival, neither PDN nor SC transplants enhanced open field locomotion (BBB) or improved sensory function. SCs but not PDN transplants resulted in an enhanced BBB subscore (SCI: 5.7 +/- 1.7; PDN: 5.5 +/- 1.5; SC: 7.6 +/- 1.3) and were better able to walk across a ladder without misstepping (SCI: 6.2 +/- 0.9; PDN: 5.6 +/- 0.8; SCs: 4.1 +/- 1.1). In sum, purified populations of SCs rather than freshly dissociated PDNs (with macrophages and myelin debris) are more beneficial as a source of cells for repairing the injured spinal cord.
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P-70 A Comparison Of Human Embryonic Stem Cell-Derived Neural Progenitor And Oligodendrocyte Progenitor Cell Transplants In Spinal Cord Injured Rats

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Spinal cord injury (SCI) is characterized by limited remyelination and locomotor recovery. Current therapeutic strategies in our laboratory include exogenous transplantation of either oligodendrocyte progenitor cells (OPCs) or neural

progenitor cells (NPCs). We have previously shown that OPCs derived from human embryonic stem cells (hESC) can significantly improve locomotor recovery following spinal cord injury, and that this recovery is accompanied by increased remyelination and neurotrophic effects of transplanted cells. Indeed, hESC-derived OPCs have been shown to secrete multiple growth factors that may be involved in increased neuron survival and branching. Neural stem or progenitor cells are also able to secrete growth factors and enhance sparing in spinal cord injured animals, but their myelinogenic capacity is relatively limited. Here, we compared the effects of hESC-derived OPC and NPC transplantation on anatomical and behavioral recovery in spinal cord injured rats. hESCs were differentiated into OPCs or NPCs and growth factor gene expressions were assessed. 1.5 million OPCs or NPCs were then transplanted into adult rats 1 week after receiving a 200 kdyne injury at T9. Two months post transplant, cell survival, remyelination, neuronal sparing and locomotor recovery was assessed in both groups. Initial results show no significant increases in remyelination or locomotor recovery in NPC transplanted animals, yet these are significantly increased in OPC transplanted animals. Additionally we have found similarities in the growth factor secretions from NPC and OPC transplants that may play a role in endogenous sparing. These data indicate that transplant-derived growth factors and sparing may not be sufficient to induce functional recovery, and suggests that remyelination is important for functional repair in spinal cord injured animals.

P-71 Neuregulin-1 Types I and III Differentially Regulate Oligodendrocyte Generation And Myelin Thickness After Spinal Cord Injury

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Axons spared after incomplete spinal cord injury (SCI) can exhibit fragmented or abnormally thin myelin, leading to slowed or failed conduction. Proper remyelination following injury is therefore a critical therapeutic target. Neuregulin-1 (NRG1) types I and III are the only growth factors shown to specifically regulate myelin thickness in development. We hypothesized that augmenting NRG1 availability following SCI could also modulate myelin thickness. Gene expression studies in the month following contusion SCI in a mouse revealed that NRG1 type III mRNA normally decreases dramatically and type I mRNA peaks early, then decreases. To test the effects of increasing NRG1 protein levels after injury, we delivered human recombinant NRG1 type I or III protein directly to the cerebral spinal fluid for 14 days following SCI. Infusion of NRG1 type I increased cellular proliferation at the lesion site but surprisingly led to thinner myelin on spared axons compared to injured controls. In contrast, infusion of NRG1 type III led to widespread increased proliferation, decreased the fraction of newly formed $NG2^+$ progenitor cells, increased the density of newly generated $Olig2^+/CC1^+$ oligodendrocytes and, importantly, led to thicker myelin on spared axons. Neither isoform significantly altered locomotor function on the Basso Mouse Scale. This work demonstrates that infused NRG1 types I and III differentially affect generation of oligodendrocytes and myelin thickness following SCI.

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P-72 Characterizing The Role Of The Astrocyte Glutamate Transporter, GLT1, In Secondary Cell Loss Following Traumatic Spinal Cord Injury

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Following traumatic spinal cord injury (SCI), there is an opportunity for preserving function by preventing/attenuating secondary white and gray matter loss. Astrocytes outnumber their neuronal counterparts approximately 10-fold, and play crucial roles in adult CNS function. In particular, astrocytes are responsible for the vast majority of CNS glutamate buffering, thereby preventing excitotoxic loss of neurons and oligodendrocytes. The present studies specifically examined the role of the major astrocyte glutamate transporter, GLT1, in secondary cell loss following SCI, and more generally are beginning to elucidate the important physiological roles played by astrocytes following CNS trauma. Using transgenic BAC (Bacterial Artificial Chromosome)-GLT1-eGFP fluorescence promoter reporter mice, region-specific spatial and temporal changes in gene and protein expression of GLT1 were found following moderate thoracic contusion SCI, including decreased numbers of GLT1-expressing cells at both the injury site and in adjacent intact tissue. These changes were accompanied by dramatic proliferation of reactive GLT1-negative astrocytes and apoptosis of GLT1-expressing astrocytes. Following thoracic crush SCI, histological and functional outcomes were worsened in $GLT1^{-/+}$ mice compared to wild-type mice, demonstrating the protective role of astrocyte GLT1 following SCI. Lastly, rats with moderate thoracic contusion SCI received intraspinal transplants of astrocyte precursors (Glial-Restricted Precursors: GRPs) engineered to overexpress GLT1 (G3s) in order to target astrocyte replacement-based maintenance of glutamate transport. Compared to media control, acute transplantation of unmodified GRPs or G3s resulted in greater spared tissue volume, decreased lesion size and increased numbers of spared neurons, and G3s promoted significantly greater benefits on these outcome measures than unmodified GRPs. These findings demonstrate the neuroprotective efficacy of transplantation-based strategies aimed at preventing secondary degeneration based on crucial astrocyte functions. This work was supported by: NIH F32-NS059155 (A.C.L.).

P-73 Differential Outgrowth And Synaptogenesis Of Corticospinal Tract Neurons In Response To Astrocytes Or Olfactory Ensheathing Cells

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A better understanding of the mechanisms that regulate the survival and outgrowth of corticospinal tract (CST) neurons will be integral in the development of novel therapeutic approaches aimed at treating diseases of the central nervous system (CNS) such as spinal cord injury (SCI), motor neuron disease (MND), and stroke. However, the in vivo complexity of these CNS diseases makes the investigation of possible therapeutics that directly affect survival or regeneration of corticospinal tract neurons extremely challenging. We use Thy1.2 transgenic mice which express yellow fluorescent protein (YFP) in postnatal day 8 (P8) corticospinal neurons as a source of CST neurons which have already established synaptic contact in the spinal cord in order to assess factors that affect survival and neurite outgrowth of axotomized CST neurons. Once in culture, the YFP-positive corticospinal neurons and interneurons represent an enriched neuronal population over glia, and they survive and extend processes over time. CST neurons exhibit differential axon extension and dendritic branch length when co-cultured with astrocytes as compared to olfactory bulb derived-olfactory ensheathing cells (OB-OECs). Given that the production of novel cortical circuitry may also underlie recovery from SCI, stroke and other neural trauma, we are now testing if differential synaptogenesis occurs in response to OB-OECs or astrocytes. This will allow us to asses CST axonal and dendritic outgrowth kinetics and to investigate whether CST neurons utilize distinct or shared pathways in promoting outgrowth and synaptogenesis. This assay system will facilitate the investigation of novel therapeutics aimed at promoting corticospinal neuron outgrowth for the eventual treatment of CNS injury and neurodegenerative disorders.

P-74 Induced Pluripotent Stem Cell-Derived Astrocytes As A Resource For Spinal Cord Lesion Repair And Plasticity

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Astrocytes represent a morphologically and functionally diverse population of cells in the central nervous system (CNS). A particular subset of these cells, reactive astrocytes, forms in response to all CNS insults, including spinal cord injury. Reactive astrocytes provide an innate defense mechanism separating spared from damaged nervous tissue, thereby limiting the extent of injury. While the formation of a scar is life preserving, the long-term maintenance of the glial scar is arguably the strongest impediment to axon regeneration and functional recovery. In contrast to injury of the adult spinal cord, embryonic injuries exhibit limited scarring and increased regeneration that can be attributed, in part, to astrocytes that not only react to injury but also provide a substrate for regeneration. Surprisingly little is known regarding the heterogeneity of astrocytes with respect to differentiation pathways and function. Our lab has spent considerable time exploring the subtypes of astrocytes and the signals that direct astrocyte differentiation from adult neural progenitor cells (NPCs). The recent achievement of generating induced pluripotent stem cells (iPS) from human fibroblasts has created a unique opportunity to investigate post-injury astrocyte behavior in a manner more directly applicable to humans. Fibroblasts are reprogrammed into iPS cells via infection with lentiviral constructs encoding *OCT4*, *LIN28*, *NANOG*, and *SOX2*. Our research tests the ability of astrocytes to be

generated from iPS cells through multiple signaling pathways (BMP4, CNTF, LIF and serum) and their differences both *in vitro* and *in vivo* with respect to neuroprotective effects. *In vitro*, these differences are demonstrated with respect to the ability of subtypes of astrocytes to buffer glutamate levels. *In vivo*, we test the astrocytes' differential capacity to support neuronal survival in a defined lesion in the rubrospinal tract, where histological observations are correlated with two behavioral assays: the pellet reaching task, and the cylinder test.
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P-75 Human Embryonic Stem Cell-Derived Oligodendrocyte Progenitor Cell Transplants Improve Recovery After Cervical Spinal Cord Injury

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Evidence that cell transplants can improve recovery outcomes in spinal cord injury (SCI) models substantiates treatment strategies involving cell replacement for humans with SCI. Most pre-clinical studies of cell replacement in SCI examine thoracic injury models. However, as most human injuries occur at the cervical level, it is critical to assess potential treatments in cervical injury models and examine their effectiveness using at-level histological and functional measures. To directly address cervical SCI, we used a C5 midline contusion injury model and assessed the efficacy of a candidate therapeutic for thoracic SCI in this cervical model. The contusion generates reproducible, bilateral movement and histological deficits, although a number of injury parameters such as acute severity of injury, affected gray to white matter ratio, extent of endogenous remyelination, and at-level locomotion deficits do not correspond with these parameters in thoracic SCI. Based on reported benefits in thoracic SCI, we transplanted human embryonic stem cell (hESC)-derived oligodendrocyte progenitor cells (OPCs) into this cervical model. hESC-derived OPC transplants attenuated lesion pathogenesis and improved recovery of forelimb function. Histological effects of transplantation included robust white and gray matter sparing at the injury epicenter, and in particular, preservation of motor neurons that correlated with movement recovery. Importantly, differential gene expression in transplanted and vehicle-treated animals at 14 and 21 days post-injury indicated that the expression of relevant, specific genes in transplanted animals deviated from the injured pattern and tended toward the uninjured pattern. These findings further our understanding of the histopathology and functional outcomes of cervical SCI, define potential therapeutic targets, and support the use of these cells as a treatment for cervical SCI.

P-76 Transforming Growth Factor α (TGFα) Alters The Astrocyte Response Following Spinal Cord Injury

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Following spinal cord injury, neural progenitor cells (NPCs) and astrocytes proliferate and migrate toward the lesion. There, the astrocytes undergo hypertrophy and contribute to the formation of the glial scar, a barrier to regeneration. Prevention of this response is deleterious to repair, demonstrating the essential role of reactive astrocytes following injury. Because of this dual role, we have proposed that targeting astrocytes with an appropriate stimulus might enhance their beneficial characteristics. Previously, we found that intrathecal administration of transforming growth factor α (TGFα) to the injured mouse spinal cord increased the infiltration of astrocytes into the lesion, accompanied by increased axon growth. However, intrathecal administration has several caveats, including activation of nonspecific targets. We hypothesized that a more direct administration of TGFα to the parenchyma surrounding the injury would result in a robust astrocyte response with limited nonspecific activation. To test this, we constructed an adeno-associated virus (AAV) that induces production of TGFα. In a short-term survival study, we found that mice injected with TGFα-AAV into the spinal cord exhibited a less-defined glial scar at the lesion site than mice injected with a green fluorescent protein (GFP)-AAV. To determine if TGFα acts on the targeted populations, NPCs and astrocytes, we examined the effects of TGFα on isolated adult spinal cord NPCs and astrocytes derived from those NPCs. We found that TGFα administration results in robust proliferation and wound closure in NPCs, but not astrocytes. TGFα elicits an elongated, bipolar morphology similar to that of radial glia in both populations. Furthermore, TGFα-treated astrocytes, but not NPCs, provide an environment supportive to dorsal root ganglion neurite outgrowth. Together, these results demonstrate that localized administration of TGFα alters the astrocyte response to spinal cord injury, and suggests that these effects may be mediated through direct actions on adult spinal cord NPCs and astrocytes.

P-77 Oligodendrocyte Genesis Occurs For Several Weeks Post-Injury Along Lesion Borders And Contributes To Axon Remyelination

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After spinal cord injury (SCI) many oligodendrocytes are damaged beyond repair and undergo apoptosis for several weeks after initial trauma. However, we and others have shown that in days following the injury, marked oligodendrocyte progenitor proliferation occurs and is followed by formation of new oligodendrocytes. These new cells are located in spared tissue and along lesion borders where they eventually outnumber pre-injury oligodendrocyte numbers. How long oligogenesis occurs and whether these cells persist to remyelinate injured axons has yet to be shown. Thus, the current study was designed to examine whether oligogenesis persists beyond the first week post-injury and if new oligodendrocytes remyelinate spinal axons. To identify new oligodendrocytes arising from proliferating

progenitors, a GFP-retrovirus was injected into the injury site on either 1, 2, 7, 14, or 21 days after moderate mid-thoracic contusion injury in rats. Animals were perfused 3 weeks after virus injection, which allowed detection of progeny cells dividing at the time of injection. Using double-label immunofluorescence, we detected new oligodendrocytes arising from cells dividing at every injection time point. This reveals that as late as 21days post-injury, endogenous spinal cord progenitors divide and subsequently differentiate into new oligodendrocytes. New oligodendrocytes integrated into spinal cord white and gray matter, especially near lesion borders. Processes from these cells were detected in contact with axons where they formed MBP+ wraps, suggesting they were remyelinating axons. To confirm oligodendrocyte remyelination after SCI, an additional set of SCI tissue was resin-embedded at different times post-injury. Analysis of this tissue confirmed the presence of oligodendrocyte remyelination as early as 21days post-injury, increasing over time through at least 10 weeks post-injury. Oligodendrocyte remyelination was especially prominent along lesion borders. These data support the hypothesis that oligodendrocytes are generated for several weeks after injury and contribute to endogenous repair processes by myelinating spinal axons.

P-78 Hydrostatic Pressure Activates An Integrin-Associated Signal Cascade In A Myelinating Co-Culture System

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Chronic nerve compression (CNC) injury occurs when peripheral nerves are subjected to mechanical forces in an ischemic environment, leading to pain, altered sensation and muscle atrophy. Early changes include demyelination with concomitant Schwann cell proliferation in the absence of axonal degeneration. Increasing evidence implicates that the extracellular matrix (ECM) acts as the transducer of injury by transmitting signals through bridging proteins such as integrins with subsequent activation of secondary messenger pathways. As heterodimeric transmembrane molecules that bind components of the ECM, integrins are attractive candidates to mediate CNC-induced changes in ECM. To better understand the pathophysiology of CNC, we sought to define the role of mechanical forces and ischemia on the ECM, and to define the secondary messenger cascades activated early in CNC. An in vitro system was designed to apply mechanical stimulus, in the form of hydrostatic compression, to myelinated neuron/Schwann cell co-cultures. Pressure, dissolved oxygen, and pH were monitored and feedback control systems were utilized to maintain homeostasis. After various durations of compression and/or ischemia, changes in extracellular components were characterized by immunolabeling various ECM components as well as specific markers of myelin, neurons or Schwann cells. Activation of integrin-associated secondary messenger pathways were assayed via Western Blot analysis of the total and phosphorylated levels of signal transduction adaptor proteins such as Paxillin and integrin-associated tyrosine kinases such as FAK, Pyk2, and Src. Exposure of co-cultures to fibronectin, known to activate integrin-associated pathways in hippocampal neurons, leads to a 3-fold increase in the phosphorylated form of Paxillin. No changes in Pyk2 and Src were detected. The effect on FAK phosphorylation appears transient, returning to baseline in 30 minutes. Preliminary experiments using the compression chamber implicate selective

activation of fibronectin-associated integrin pathways in response to mechanical compression.

P-79 Evaluation Of Substrate Mechanics And Myosin II Inhibition On Cortical Neuron Outgrowth

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A substantial amount of literature has shown significant effects of substrate mechanics on cell migration, morphology and behavior for an abundance of cell types. Recently, an emphasis has been placed on the effects of substrate stiffness on neurons, in an attempt to optimize the development of efficient biomaterials for neural regeneration. To date, published studies have revealed contrasting effects of substrate compliance with neurite outgrowth, with some reporting increases in the rate of outgrowth and branching with decreased gel stiffness, while others show no differences in length or decreases in outgrowth and branching. Here, we characterize polyacrylamide gels ranging in stiffness between 900Pa – 13kPa and explore substrate compliance effects on single cell cortical neurite outgrowth. Surprisingly, we report no significant differences in process extension lengths during the first 72 hours for neurons cultured on both poly-d-lysine (PDL) and laminin-coated substrates. Consistent with this observation, we observe outgrowth dynamics for individual processes and report a similar extension rate for all stiffnesses. In addition to process length, the number of neurites and the average neurite length are also insensitive to stiffness. Comparisons of PDL versus laminin outgrowth show more directed process extension, more dynamic extension and retraction behavior, and greater cell body displacements on laminin. Lastly, we study the role of myosin II (MII) during process extension dynamics, which include periods of extension, rest, and retraction. Different effects of MII inhibition occur during these outgrowth stages, with MII inhibition increasing axon extension during the resting periods for both PDL and laminin-coated substrates. However, during periods of extension, MII inhibition decreases the extension rate on all substrates. Collectively, this data illustrates an independence of substrate stiffness on cortical outgrowth, and a potential method to encourage axonal extension from a previously stationary state through the use of MII inhibition.

P-80 Afferent Plasticity In SCI: Neurophysiological Assessment Of Cutaneous Nociceptive Input Mediated Dysautonomia In The Rat

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Spinal cord injury (SCI) can result in autonomic dysfunction due to disassociation of spinal sympathetic neurons from supraspinal centers. Clinically, this can result in autonomic dysreflexia, a dangerous rise in blood pressure (BP) in response to nociceptive input from either visceral or cutaneous afferents. Animal models have been developed where colonic distention or skin pinch after SCI can

cause a marked increase in BP and that has been correlated with sprouting of nociceptive afferents. There have been few studies, however, that examine the neurophysiology of post-SCI dysautonomia. Specifically, we do not know the necessary and sufficient amount or location of nociceptive afferent activity that produces dysautonomia. We have been studying neural plasticity in a cutaneous nociceptive intersegmental spinal reflex after SCI and are investigating how that plasticity might affect autonomic function. The cutaneus trunci muscle (CTM) reflex produces a skin "shrug" in response to pinch on a rat's back and is mediated by a three neuron circuit: C and A-delta afferents in segmental dorsal cutaneous nerves (DCNs), ascending propriospinal interneurons, and the CTM motoneuron pool. Under pentobarbital anesthesia, we have monitored BP changes via carotid artery cannulation in response to stimulating the segmental DCNs from L1 to T6 at various frequencies (0.2, 0.5, 1, 2, 5, and 10 Hz) at C fiber strength in both normal Long Evans rats and those that have undergone either cervical or thoracic SCI. In normal animals, stimulation causes a depressor effect on BP in a stimulation frequency and DCN level specific manner. In animals with SCI, stimulation of DCNs above the level of injury produces the same BP changes as in uninjured animals. Stimulation of DCNs below the level of injury, however, no longer causes a depressor effect and can cause a pressor effect, again in a stimulation frequency and DCN level dependent manner.

P-81 The Effect Of Housing In An Enriched Enviornment On Axonal Sprouting, BDNF Expression And Synaptic Plasticity In Rats That Receive A Midthoracic Spinal Cord Hemisection

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Previous studies have shown that activity-based therapies improve hindlimb motor function in spinally hemisected rodents. There is evidence that recovery of hindlimb motor function is mediated by brain derived neurotrophic factor (BDNF) and synaptic plasticity below the lesion. However, there is recent evidence that axonal sprouting from descending pathways may also play a role in hindlimb motor recovery. In this study, we examine the effect of housing in an enriched environment (EE) on sprouting in the corticospinal tract, BDNF expression and synaptic plasticity in the lumber spinal cord of rats that received a spinal cord hemisection. Biotinylated dextran amine was injected into the right motor cortex in 24 rats. One week later, the rats received a lateral spinal cord hemisection at the midthoracic level. Half of the rats were housed in an EE while the other half received standard housing. Based on preliminary analyses, both groups of rats recovered movement in the impaired hindlimb 1-2 weeks after hemisection. Further experiments will be performed to determine if housing in an EE 1) improves behavioral recovery 3-4 weeks after hemisection; 2) enhances BDNF and synaptophysin expression in the lumbar spinal cord and 3) enhances sprouting of the contralateral corticospinal tract. The findings from this on-going study may provide insight into the mechanisms of activity-based therapies used to improve function following spinal cord injury.

P-82 Late Effects Of Enriched Environment (EE) And Multi-Modal Early Onset Stimulation (MEOS) After Experimental Traumatic Brain Injury: Ongoing Neurofunctional Improvement Despite Sustained Lesion Volume

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We have demonstrated that the combination between MEOS and EE applied to rats for 7-15 days after experimental traumatic brain injury (TBI) in the rat was associated with reduced CNS lesion volume and enhanced reversal of neuromotor dysfunction. In a continuation of this work, we tested whether these effects persisted for longer post-operative periods, e.g. 30 days post-injury (dpi). Rats were subjected to lateral fluid percussion (LFP) or sham injury. After LFP, one third of the animals (injured and sham) was placed under conditions of standard housing (SH), one third was kept in EE-only, and one third received EE+MEOS. Standardized composite neuroscore (NS) for neurological functions, rotarod and computerized analysis of the vibrissal motor performance were used to assess post-traumatic neuromotor deficits. The Barnes Ciurcular Maze test was used to assess neurocognitive impairment and recovery. These were followed by evaluation of the cortical lesion volume (CLV) after immunostaining for neuron-specific enolase (NSE), caspase 3 active, and GFAP. Finally, the volume of cortical lesion containing regeneration-associated proteins (CLV-RAP) was determined in sections stained for GAP-43, MAP2, and neuronal class III beta-tubulin. We found (i) no differences in the vibrissal motor performance; (ii) EE+MEOS rats performed significantly better than SH rats in NS; (iii) EE-only and EE+MEOS animals, but not SH rats, showed better recovery at 30 dpi than at 15 dpi; (iv) no differences among all groups in CLV (larger than that at 15 dpi) and CLV-RAP, despite a clear tendency to reduction in the EE-only and EE+MEOS rats. We concluded that EE+MEOS retards, but cannot prevent the increase of lesion volume. This retardation is sufficient for a continuous restoration of neurological functions. This study was supported by the Koeln-Fortune Program, University of Cologne (Germany)

P-83 Cortical Astroglial Subpopulation Inhibits Axon Growth *In Vitro*

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Astroglia cells are the most heterogeneous and predominant glial cell type in central nervous system, the functional significance of this heterogeneity is largely unknown right now. Damaged astrocytes showed inhibition to axon regeneration *in vivo* and *in vitro*, but primary astrocytes were commonly considered good supportive substrate for neuronal attachment and axon regeneration. The reason for this discrepancy is unknown. In the present study, the influence of astrocytes heterogeneity on neuron attachment and neurites regeneration was examined in vitro.

Mixed glial cell culture from neonatal cortices was co-cultured with purified dorsal root ganglia neurons. Consistent with previous studies, majority astrocytes were supportive to neuron attachment and axon growth. To our surprise, we observed subpopulation astrocytes showed strong inhibition for neuron attachment and axon growth. These cells grew clustery and formed substructures with variable sizes and shapes, regenerating axons stopped abruptly on encountering these substructures, axons growing on them showed dystrophic appearance, and neurons located atop of these substructures showed few short axon regeneration or did not show axon growth at all, but neuronal survival was unaffected. Immunocytochemistry study showed these inhibitory substructures were formed by GFAP positive cells. To our knowledge, this is the first time showed the existence of cortical subpopulation astrocytes with anti-neuron attachment and inhibition axonal growth property. Identification of this new type of inhibitory astrocytes subpopulation may lead into deep insight on the complex cellular and molecular mechanism of axonal regeneration failure.

P-84 Trauma-Activated T-Cells Are Required For NT-3-Induced Axonal Sprouting In The Injured Spinal Cord

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Over-expression of Neurotrophin-3 (NT-3) induced axonal sprouting in acutely, but not chronically, injured spinal cords (SC). However, NT-3 could induce axonal sprouting in rats with chronically injured SC if immune responses were re-activated with lipopolysaccharide. The degree of axonal sprouting coincided with the number of $CD4^+$ T-cells present in the SC suggesting that they were involved. To test this we compared axonal sprouting in athymic nude rats (rnu/rnu) that lack functional T-cells and in normal controls (rnu/+) that have them. Two weeks after a unilateral corticospinal tract (CST) lesion at the level of the medulla, NT-3 was over-expressed in the lumbar motoneurons contralateral to the spared CST with an adenoviral vector carrying the NT-3 gene (Adv.NT-3). Adv.LacZ was the control vector. At 35 days post-lesion we measured axonal sprouting from the unlesioned CST across the midline to the lesioned side at the lumbar level. The number of axons sprouting in response to NT-3 was greater in the rnu/+ rats compared to rnu/rnu rats. There was no significant difference in axonal growth between the rnu/+ rats treated with Adv.LacZ and the rnu/rnu rats treated with Adv.NT-3 or Adv.LacZ. When we grafted T-cells from uninjured rnu/+ rats into rnu/rnu recipients NT-3 did not enable axonal sprouting but, when we grafted $CD4^+$ T-cells isolated from rnu/+ rats that had received CST lesions NT-3 induced axonal sprouting. These findings suggest that $CD4^+$ T-cells activated by trauma-associated antigens participate in NT-3 induced axonal growth in our model. Supported by the Christopher and Dana Reeve Foundation and the Craig H. Neilsen Foundation.

P-85 Apolipoprotein E-Derived Peptides Improves Anatomical And Locomotor Recovery After Rat Spinal Cord Injury

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Apolipoprotein E (apoE), a well known plasma lipoprotein for its important role in lipid and cholesterol metabolism, has also been implicated in Alzheimer's diseases and neuroprotection after experimental autoimmune encephalomyelitis and cerebral ischemia. In this study, we tested the therapeutic efficacy of an apoE-mimetic peptide, COG112 in a clinical relevant rodent spinal cord injury (SCI) model. Rats received a moderate mid-thoracic contusion and were randomly divided into groups receiving saline, control peptide COG339, or apoE peptide COG112. Peptide or saline was injected intravenously at 15 minutes post-injury and then intraperitoneally twice a day for the first 7 days post-injury. The injured spinal cords were analyzed histologically at 7 and 56 days post-injury. Locomotor function was assessed using the Basso-Beattie-Bresnahan (BBB) score and Treadscan. Our results showed that COG112 significantly decreased the inflammation in the injured spinal cord at 7 days post-injury compared to saline- or COG339-treated group. It also significantly decreased the injury size and increased the spared white matter at the injured epicenter, 0.6 mm rostral and caudal to epicenter at both 7 and 56 days post-injury. Importantly, BBB scores were significantly greater in COG112 treated animals compared to other two groups from week 1 to week 8 post injury. Significantly improvement in toe spreads was also noted by Treadscan analysis. COG112 significantly inhibited the inflammation-induced downregulation of the tight-junction proteins in the in vitro blood-brain-barrier model and restored its transendothelial electrical resistance and permeability. Evaluation of its effects on the permeability of blood-spinal cord-barrier (BSCB) after contusion is undergoing. Thus, our results demonstrate that treatment with apoE peptide enhances the locomotor functional recovery and decreases the neural tissue loss likely by protecting BSCB and inhibiting the inflammation after SCI, suggesting that that apoE peptide may be a novel and promising neuroprotective reagent for SCI.

P-86 Administration of The PPAR-A Agonist Gemfibrozil Deteriorates Functional and Anatomical Outcomes Following Spinal Cord Injury (SCI)

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Peroxisome proliferator activated receptors (PPAR) are nuclear hormone receptors known for their role in lipid metabolism. PPAR-α, an isotype of the PPAR family, has been shown to modulate neuroinflammation. Agonists of PPAR-α improve clinical scores in models of multiple sclerosis and other neurodegenerative diseases. Spinal cord injury (SCI) afflicts >11,000 individuals annually in United States with no accepted therapeutic treatments. SCI has a large inflammatory component involved in lesion progression and altering this response with agonists of PPAR-α could lead to better functional recovery. Based on this hypothesis, we studied the effect of oral treatment of a specific PPAR-α agonist gemfibrozil (Lopid ™) on SCI mice. Since gemfibrozil is an FDA-approved medication it could be rapidly

adapted for SCI treatment. In this study, animals were pre-treated 3 days before injury with drug or vehicle and survived for 3d post-injury (dpi), 7dpi or 28 dpi. The Basso Mouse Scale (BMS) was used to assess locomotor abilities. Surprisingly, the behavioral assessment revealed that gemfibrozil group had more severe behavioral deficits compared to vehicle group starting at 5 dpi continuing through end of the study. Anatomical analysis of eriochrome cyanine/neurofilament-stained tissue used for delineating spared tissue showed a significant reduction in spared white matter in gemfibrozil animals versus controls. While there were no changes in the extent of macrophage activation, gemfibrozil decreased astrocytic immunoreactivity (GFAP) and displayed a trend for decreased T-cell infiltration (CD3+ cells) at 28 dpi. Thus, in stark contrast to other neuroinflammatory diseases where PPAR-α agonists are beneficial, gemfibrozil is particularly detrimental after SCI and could potentially exacerbate conditions of patients already on gemfibrozil. Current studies are examining whether post-treatment of gemfibrozil after SCI in mice is beneficial. Overall, the current results are important from a clinical perspective where the same drug can have contrasting effects in different disease models.

P-87 Combined VEGF And Angiopoietin-1 Gene Transfer Using AAV Vectors After Spinal Cord Injury

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A consequence of spinal cord injury is the disruption of spinal vasculature, and it is this disruption that contributes to the initiation of cascade of biochemical events leading to secondary damage from the ischemic and inflammatory responses. A possible therapeutic intervention focuses on promoting angiogenesis to restore the disrupted vasculature. Angiopoietin-1 (Ang-1) and vascular endothelial growth factor (VEGF) play an important role in vascular formation and maturation, indicating that these agents may be promising for SCI. Adeno-associated virus (AAV) engineered to express VEGF or Ang-1 were injected in combination into the lesion epicenter following a moderate-severe contusion injury at thoracic level 7. Our data indicates that the combined administration of AAV-VEGF and AAV-Ang-1 results in reduced lesion volume by magnetic resonance imaging compared with viral controls. Dynamic contrast-enhanced (DCE) imaging, performed to examine the effect viral treatment had on the blood spinal cord barrier permeability, indicated a significant increase in the amount of non-enhancing area in VEGF/Ang-1 treated animals compared with viral controls in the region rostral to the lesion epicenter. The open field locomotor BBB assay indicated a significant improvement in combined viral treated animals compared with viral controls. Western analysis of the lesion epicenter indicated a significant increase in viral vectors Ang-1 and VEGF protein expression in the chronic phase of injury. Spinal inflammation was assessed through quantification of microglia, determined by the expression of Iba-1, a calcium-binding protein, infiltrating into the lesion epicenter. Westerns analysis of Iba-1, indicated no significant difference in microglial recruitment in injured viral-treated versus injured-control subjects indicating that viral treatment did not elicit a greater inflammatory response compared with viral control and saline treated groups. This study indicates that viral gene delivery promoting angiogenesis in combination with vessel maturation may be a promising therapeutic candidate for SCI treatment.

P-88 Vascular Endothelial Growth Factor – A Novel Player In SCI Pain

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Vascular endothelial growth factor (VEGF) is an essential promoter of angiogenesis in response to injury and has been shown to have multiple effects after damage to the central nervous system (CNS). It is hypothesized that administration of VEGF following CNS trauma stimulates the growth of new vasculature to improve perfusion and provide nutritional support to the tissue. Studies have indicated that VEGF not only encourages vessel growth, but it also results in neuroprotection and axonal outgrowth. Accordingly, we have found that acute intraspinal administration of VEGF following contusive spinal cord injury (SCI) results in tissue sparing as seen with magnetic resonance imaging (MRI) studies; however, we have observed a novel finding that there was significant increase in persistent, chronic pain, using the von Frey assay, in rats receiving VEGF compared with vehicle controls. We observed that 33.33% of rats receiving VEGF treatment developed persistent pain, while 9.09% of anti-VEGF and 7.69% of vehicle-control rats showed signs of the onset pain. Our results also indicated a direct association between VEGF treatment triggering chronic pain, a significant increase in axons in the dorsal columns and dorsal horns as well as a significant increase in the protein levels of Calcitonin Receptor-Like Receptor (CRLR). These findings indicate that treatment with VEGF in the acute phase of SCI results in tissue sparing; however it may also encourage non-specific sprouting of axons into the spinal cord areas associated with pain transmission.

P-89 Intermittent Hypoxia: A Novel Treatment To Induce Spinal Plasticity

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Intermittent hypoxia (IH) initiates serotonin-dependent plasticity, particularly in the respiratory motor control system. One well studied model of IH-induced spinal, respiratory plasticity is phrenic long-term facilitation (pLTF). In brief, pLTF requires serotonin-dependent BDNF synthesis on or near phrenic motor neurons. Subsequent activation of the high affinity BDNF receptor, TrkB, is sufficient to induce pLTF. Since pLTF is enhanced by pre-conditioning with repetitive IH, we hypothesized that repetitive exposure to acute intermittent hypoxia (AIH) would enhance spinal expression of molecules that play a critical role in pLTF, including serotonin, 5-HT2A receptor, BDNF and TrkB. Thrice-weekly exposure to AIH (3xwAIH; 10x5 min episodes per day, 10.5% O2, 5 min normoxic intervals) for 4 weeks enhanced AIH-induced pLTF in anesthetized Lewis rats vs. normoxic controls (176±42% vs. 55±8% at 60 min post-hypoxia, respectively; $p<0.01$; n=10 per group). After 3xwAIH (10 weeks) in Sprague-Dawley rats, serotonergic terminal density and 5-HT2A receptor expression increased in the phrenic motor nucleus at C4. BDNF and TrkB immunoreactivity were both upregulated in presumptive phrenic (and non-respiratory) motor neurons. 3xwAIH also increased phosphorylation of extracellular-signal

regulated kinase (p-ERK) and protein kinase B in presumptive phrenic motor neurons. Since these molecules are required for the initiation and/or maintenance of AIH-induced pLTF, we hypothesize that changes in their expression within phrenic motor neurons underlie pLTF enhancement. There was no evidence for reactive gliosis or cell death in the hippocampus following 3xwAIH, suggesting that this mild protocol is not associated with adverse side effects caused by more severe protocols of intermittent hypoxia. Repetitive AIH may be a useful therapeutic treatment to increase respiratory motor output in cases of impaired ventilatory capacity. For example, repetitive AIH is a promising treatment for respiratory insufficiency following cervical injuries or during neurodegenerative disease. (Supported by NIH HL080209, HL69064 and NS057778).

P-90 A New Animal Model Of Motor Neuron Loss: Selective Neuronal Deficits After Intraneural Injection Of *Ricin*

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The goal of the present study was to create a new model of motor neuron loss and use it to test motor neuron cell replacement therapies. We produced weakness and loss of function by damaging the sciatic nerve with an injection of the toxic lectin *Ricinus communis* agglutinin I (RCA I or ricin). Axonally-transported toxins like ricin make selective lesions of the nervous system. Since the sciatic nerve supplies sensation, reflexes and movement to most of the lower limb, damaging this large nerve models the weakness and paralysis that occur in spinal cord injury and motor neuron disease. We used motor, sensory-motor, locomotor and reflex-based tests (inclined plane, balance beam walking, sections of the BBB scale, kinematic analysis and toe spreading reflex) to measure loss of function after ricin injection. All of these tests showed a significant and long-lasting difference between ricin-injected and sham-injected animals. Loss of function was also demonstrated by decreased retrograde transport and direct measurements of muscle wasting. We show histochemical and molecular evidence of sciatic nerve damage (loss of axons and cell bodies, apoptotic cell death). This battery of tests documents the extent of the ricin-induced damage and provides a baseline that can be used to judge efficacy of motor neuron progenitor transplants in preclinical trials. Towards this aim, we have transplanted embryonic stem cell-derived motor neuron progenitors (MNP) into the spinal cord of these ricin-lesioned animals. Transplanted MNP survive in the spinal cord for at least six months. Continued work focuses on characterizing changes in the transplanted cells and monitoring functional outcomes.

P-91 Novel Astroglial Cell Subtype In Spinal Cord Shows Strong Axon Growth Inhibition And Antiadhesion Property *In Vitro*

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Astrocytes are heterogeneous cell population, but this heterogeneity functional significance is still elusive. Astrocytes heterogeneity influence on axon growth was addressed by adding purified dorsal root ganglia (DRG) neurons to neonatal rat spinal cord mixed glial cells. A special glial cell population named glia^{stop} showed strong neurite growth inhibition and anti-adhesion activity for DRG neurons. Glia^{stop} cells were characterized by monolayer, smooth cell surface with no other cells growing on top. Other astrocytes were characterized by multiple-layer cells, coarse surface with compact and high density small size oligodendrocytes. Few neuronal attachments occurred on glia^{stop} cells and neurons settled on glia^{stop} cells showed no or very short, stiff neurites growth but could survive more than 20d without any signs of death or degeneration. Neurons attached on oligodendroctyes enriched astrocytes grew out neurites within 3h. Neurites growth stopped and turned around to avoid extending into glia^{stop} region. Constrained neurites growing on oligodendrocytes enriched region formed high density network and formed whirl like structure. These glia^{stop} cells were GFAP $^{low/-}$, S100^{-}, OX42^{-}, fibronectin^{-} and Thy1^{-}, which were specific markers for astrocytes, schwann cells, microglia, meningela and fibroblast cells. These glia^{stop} were quite different from classical axon growth supporting GFAP+ astroglial cells. This data, for the first time, showed that the astrocytes heterogeneity of spinal cord had different influence on axon regeneration. Understanding glia^{stop} cells inhibition mechanism will offer new information about the function of astrocytes heterogeneity, provide new clues for axon growth and regeneration.

P-92 Control Of The Scarring Process And Enhancement Of Intrinsic Growth Capacity: Dual Effect Of Taxol On Spinal Cord Injury

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A major concern in spinal cord injury is the development of an abnormal hypertrophic scar that occurs in lesioned tissue, thereby compromising axonal regeneration and repair. This scarring process, mainly driven by exacerbated TGF beta signalling, leads to the formation of a lesion site that is dominated by connective tissue and surrounded by a dense astroglial scar. Reactive astrocytes and infiltrating cells produce abundant extracellular matrix posing both a physical and a molecular barrier to the regrowth of severed axons. In this study, we explored whether a local stabilization of microtubules with taxol can control the aberrant scarring induced by spinal cord injury. We found that low doses of taxol infused directly in the lesion site decreases the accumulation of connective tissue. This effect is accompanied by marked reduction of extracellular matrix components including fibronectin and proteoglycans. Moreover, we provide evidence that taxol treatment dampens the Smad-dependant /TGF beta signalling pathway both *in vivo* and *in vitro*, supporting the notion that taxol and microtubule stabilization can act on the scarring process "*per se*". Importantly, we could also show that the same treatment enhances the intrinsic growth potential of postnatal neurons plated onto growth inhibitory substrate.

We therefore believe that taxol, by promoting intrinsic axon growth capacity on one hand and reducing the growth inhibition of the scar on the other hand, offers in one molecule a combined therapy, a requirement that is essential for successful regeneration.

P-93 Directing Axon Regeneration After Spinal Cord Injury

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In this study, we examined the hypothesis that increasing the level of neuronal cAMP combined with degradation of chondroitin sulfate proteoglycans and creating a growth supportive pathway in the spinal cord can enhance the regeneration of supraspinal neurons and promote function recovery. A unilateral hemisection was made at the cervical level of the spinal cord. Immediately after lesion, rats were randomly divided into four groups. In group 1, minipumps were implanted subcutaneously to deliver rolipram (0.5mg/kg/day) or DMSO for two weeks. In Group 2, lentivirus encoding chondroitinase or GFP (Chase-LV or GFP-LV) was injected at rostral and caudal stumps. In Group 3, lentivirus encoding NT-3 or GDNF (NT-3-LV or GDNF-LV) was injected at the rostral and caudal stumps of the lesion. In Group 4, rats were received a combined treatment with rolipram, Chase-LV and NT-3-LV or GDNF-LV. Animals survived for four weeks. Regeneration of rubrospinal (RST) and raphespinal tract were examined by BDA staining and serotonin staining (5-HT). Cylinder test was performed for behavioral recovery. In both rolipram and Chase-LV treatments, RST axons grew to the lesion border but none grew into the lesion site. 5-HT axons grew into the lesion area but not through the lesion. In NT-3- and GDNF-LV group, some RST axons grew into the lesion site but not through the whole lesion area. In combined treatments, more RST labeled axons were found at the lesion border in rolipram/Chase/GDNF-LV group. Behavioral improvement was found in some rats in both combined treatment groups but not in the single treatment group. This study suggests that multiple combined treatments can promote axon regeneration and improve some function recovery. Future studies will test effects of delayed treatment on promoting axon growth and functional recovery or use a chronic SCI model for multiple treatments (supported by PPA107969 to YJ).

P-94 Targeted Gene Transfer Rescues Sleep Behavior in a Neurodegenerative Mouse Model

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Narcolepsy is a neurodegenerative disorder linked to the loss of orexin neurons. Gene transfer has proven to be an effective neurobiological tool in a number of neurodegenerative diseases but it is not yet known if it can also correct a sleep disorder. Here we constructed a replication-defective herpes simplex virus-1 (HSV-1)

amplicon-based vector to test if orexin gene transfer could reverse the symptoms of narcolepsy in orexin knockout mice with narcolepsy phonotype. An HSV-1-PrpUC vector containing mouse prepro-orexin gene and eGFP was delivered into the lateral hypothalamus (LH) of orexin knockout mice (n=13) and sleep-wake behavior including narcoleptic attacks were monitored. Control mice (n=9) were injected with vector carrying solely the reporter gene (GFP). Sleep was also recorded from wildtype (WT) mice (n=9) of the same background strain (C57BL/6J) and age (3-7 months old; 20-35 g) as the orexin knockouts. Sleep was measured during the 2nd and 4th days post-injection. Numerous orexin-A immunoreactive neurons in the LH of orexin knockout mice were evident 1-3 days after gene transfer followed by a decline after the 4th day. ELISA assay detected the orexin peptide in the CSF of orexin KO mice given the orexin gene indicating synthesis and release of orexin from neurons. Orexin gene transfer into the LH decreased the incidence of cataplexy by 60% (versus control vector), and the levels of REM sleep during the second half of night were same as WT. This is the first evidence that a sleep disorder, such as narcolepsy, can be improved by gene therapy. This is "proof-of-principle" that a complex behavior such as sleep can be rescued by targeted gene therapy.

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P-95 Ketogenic Diet Initiated After SCI Improves Functional Recovery in Rats

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The purpose of this study was to evaluate the effects of a simple dietary intervention to boost functional recovery after acute SCI. We investigated the ketogenic diet (KD), a diet low in carbohydrates and high in fat. As during fasting, this withholding of carbohydrates forces the body to use fat as fuel. KD has been well recognized as an effective non-pharmacological therapy for drug-resistant epilepsy. Studies in animals during the past decade have shown that KD may also be beneficial in certain neurodegenerative diseases including brain injury, Alzheimer's and Parkinson's disease. Somewhat surprisingly, no studies have examined the effects of KD after acute SCI.

We used a controlled cervical spinal cord contusion model in rats to study the role of KD after SCI. Immediately after the impact, the rats started either a standard diet or KD. As a result of this injury, the rats fed a standard diet are profoundly affected in their ability to use the injured front paw while rearing, and almost exclusively use their non-impaired forelimb. In contrast, KD resulted in a large improvement in the ability of these animals to use their injured paw after 5-7 weeks of treatment. At a follow-up of 14 weeks, 54% of the animals showed a 15-fold more frequent usage of the affected paw than the rats on a standard diet. We found similar positive effects of KD after partial crush injury of the cervical spinal cord emphasizing the robust nature of the effect of this diet.

Our results suggest that KD might be an appropriate initial treatment to improve outcomes in human spinal cord injuries. Since KD is already a well-established therapy for epilepsy and ketogenic formulas have already been developed for clinical use, this diet could be readily translated into the clinical setting of spinal cord injury.

P-96 The Therapeutic Effect of Netrin-1 Overexpression via AAV Gene Transfer in Experimental Stroke

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In addition to their role in axon guidance during CNS development, netrins have been implicated in angiogenesis and neuroprotection. The aim of this study is to determine whether overexpression of Netrin-1 via adeno-associated viral (AAV) gene transfer confers neuroprotection following experimental stroke. Stroke was induced unilaterally by the distal MCAO method under isoflurane/O_2/N_2O anesthesia. 1×10^{10} genome copies of AAV-Netrin-1 (replication-incompetent vector encoding chicken Netrin-1) or AAV-LacZ were injected medial and posterior to ischemic lesion in the rat ipsilateral sensori-motor cortex at 3 days following dMCAO. Transgene expression was analyzed by RT-PCR, immunohistological staining and western blotting using antibody to chicken Netrin-1. Double immunofluorescence staining was performed to determine the cell type specific expression of the endogenous and transduced Netrin1 at various time points following vector injection or dMCAO. Infarct volume was determined at 3 weeks following gene transfer. Our data indicate that endogenous Netrin-1 was induced in the neurons in the peri-infarct regions during the first week after MCAO. Following AAV-NT1 gene transfer, transduced Netrin-1 immunoreactivity was also found mainly in the neurons in the peri-infarct regions. The expression of transduced Netrin-1 began at 1 day and plateaued about 3 weeks following vector injection. Although rats received Netrin-1 gene therapy in general had a smaller infarct volume as compared to those received AAV-LacZ, the difference was not significant (AAV-LacZ: 8.5± 0.8x10^{10} mm^3, AAV-Netrin-1: 6.7± 0.7x10^{10} mm^3, p=0.085). In summary, both ischemia-induced endogenous and transduced Netrin-1 were found in the neurons of the peri-infarct regions. However, overexpression of Netrin-1 via AAV gene transfer at 3 days following MCAO did not significantly reduce the infarct size. Ongoing investigation will further determine the optimal therapeutic window for AAV-Netrin-1 gene therapy and its effect on angiogenesis, vascular permeability, CST axonal remodeling and gait function recovery following experimental stroke.

P-97 Analyses Of Lesion Volume, Demyelination And Neuronal Injury Following Stereotaxic Radiofrequency Lesioning In Spinal Cord And Brain

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Radiofrequency (RF) lesioning is used in clinical neurosurgery. RF lesions are caused by induced thermal and electrical fields. RF may have value as a lesion method in primate spinal cord injury studies because it can be precisely localized without extensive surgical exposure. Some data indicates that RF lesions cause

extensive axonal demyelination. To address these issues more thoroughly than is possible in primates we determined whether RF caused reproducible dose-tissue injury responses, demyelination and neuronal injury in rodent CNS. We tested whether ex vivo 4.7 Tesla (T) MRI or histological analysis provided more accurate measurement of lesion size and peri-lesional demyelination. Methods: Animals received brain and spinal cord RF lesions. Temperatures tested were 50, 60, 70 and 80°C, RF delivery times were 60 or 80 seconds. Thirty days after injury, rodents were perfused. Specimens underwent ex vivo 4.7T MRI. Sections were stained with H/E - Luxol fast blue. Demyelination was assessed using neurofilament and myelin basic protein immunolabeling. Gliosis was studied with GFAP. Volumetric analysis compared histologic and MRI sections and using 3D reconstruction software. Results: Lesions had ellipsoidal geometry with central necrosis and an inflammatory reaction. Cavity volume was proportional to temperature ($R^2 = 0.92$), with insignificant cavities at 50°C and destruction of gray and white matter at temperatures ≥60°C. Reactive astrogliosis was found in the transition zone between the cavity and spared tissue. Histological analysis allowed a more precise estimate of injury volume than MRI. Demyelination was present in a narrow tissue zones in the periphery of cavities. Unexpectedly, neuronal injury was found to extend further from injury epicenters than myelin injury. Conclusions. RF induced lesions of the CNS are reproducible, causing both myelin and neuronal injury. Current MRI techniques are still less accurate for measurement of injury volume than histological analysis.

P-98 Comparative Microarray Analysis Following Traumatic Brain Injury And Stroke

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The pathophysiological processes that follow TBI are very similar to those observed following ischemic stroke and other acute brain injuries (ABIs). These include damage to the blood brain barrier, release of excitatory amino acids, free radical generation, cerebral edema, reduced cerebral blood flow, hypoxia, ischemia, and inflammation. The pathological similarities between TBI and other ABIs infer that effective neuroprotective strategies for one may also be effective for the others. In order to develop such strategies, it is necessary to understand and compare the gene expression patterns resulting from these neurological insults. By understanding the common and specific pathological pathways initiated by the lesions, targeted treatment protocols can be developed to counteract the ensuing deleterious processes. Previous work in our laboratory revealed that there were significant differences between the gene expression patterns following transient and permanent middle cerebral artery occlusion (tMCAO and pMCAO, respectively) stroke models, indicating distinct mechanisms for neuronal cell death for each model. Additionally, we have shown that neuregulin-1 is neuroprotective for both tMCAO and pMCAO. The goal of this study was to assess gene expression profiles after TBI and show how it compared to expression in the MCAO models. Adult Sprague-Dawley rats received a unilateral controlled cortical impact using the Pittsburgh Precision Instruments, Inc. device. We sacrificed the rats 24 hours post-injury and prepared the brains for microarray analysis. After dissection and RNA extraction, biotinylated cRNA was

hybridized to an Affymetrix Rat Genome 230 2.0 GeneChip and scanned according to manufacturing guidelines. Genespring and Ingenuity Pathway Analysis data analysis tools were used to compare and contrast gene expression profiles in TBI and MCAO stroke models. Based on this information, we proposed potential neuroprotective strategies that may be effective in one or more models of ABI. Specifically, we looked at whether neuregulin-1 projects to be neuroprotective for TBI.
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P-99 Novel Compounds That Overcome Inhibition Of Cns Regeneration

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Following traumatic insults to the brain or spinal cord, axons in the central nervous system (CNS) largely fail to regenerate. These insults lead to the deposition of myelin debris and the formation of a glial scar, together comprising a large number of regeneration inhibitors. Prominent among these is a family of astrocyte-derived chondroitin sulfate proteoglycans (CSPGs). Attempts to achieve regeneration of long axon tracts by overcoming inhibitory signals from myelin and CSPGs have met with limited success, partially owing to a lack of detailed knowledge about the signaling mechanisms underlying inhibitory signals. One way to advance this knowledge is to identify chemical compounds that can overcome regeneration inhibition.
Our lab has undertaken a neuronal phenotype-based screen of a library of novel triazine compounds, to identify those that promote neurite growth on inhibitory substrates. We identified four “hit” compounds based on their ability to increase neurite outgrowth on a substrate of CNS myelin. Interestingly, these compounds also increase neurite outgrowth from several different classes of CNS neuron on a substrate of inhibitory CSPGs, suggesting that they act through a common signaling pathway. Most excitingly, one of the compounds, F05, promotes acute regeneration of severed dorsal column axons, and promotes regeneration of crushed optic axons *in vivo*. The compounds appear not to act through cAMP, protein kinase C, or the EGF receptor. However, compound F05 alters microtubule dynamics in cultured neurons, suggesting a potential mechanism through which regeneration could be affected. Elucidation of the mechanism(s) of action of these compounds should provide insight into mechanisms of regeneration inhibition, and may lead to novel therapeutic strategies for CNS injury.

P-100 From Biomarkers To Therapeutic Targets: Lipid Peroxidation Products In Spinal Cord Injury

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Lipid peroxidation (LP) aldehydic byproducts such as 4-hydroxy-2-nonenal (4-HNE) and acrolein are elevated in spinal tissue following spinal cord injury (SCI) and appear to damage cellular functions including mitochondrial respiration by forming adducts with various proteins. However, the direct contribution of LP byproducts to posttraumatic mitochondrial impairment, is unclear. We initially investigated the temporal relationship of 4-HNE in the contusion-injured rat spinal cord (Infinite Horizons device, 200 kdyn, T10) using immunohistochemistry. Protein-bound 4-HNE increased substantially at 3hr post-SCI in the injury epicenter and staining was observed 6-9mm in the rostral or caudal directions in neurons and microvessels. The 4-HNE staining spread outward by 24 and 72 hrs and dissipated 1-2 weeks following SCI. We then assessed the direct effects of 4-HNE and acrolein on mitochondria isolated from spinal cords of naïve rats. Ficoll gradient-isolated mitochondria from rat spinal cords (and brains) were incubated with carefully selected doses of 4-HNE or acrolein followed by measurement of complex I and complex II-driven respiratory rates. Both compounds impaired mitochondrial respiration in a dose-dependent manner. 0.1μM 4-HNE significantly compromised mitochondrial respiration, whereas acrolein was 10x more potent, significantly impairing mitochondrial respiration at 0.01μM concentration. In comparison, 10-fold higher concentrations of both 4-HNE and acrolein were necessary to impair brain mitochondria isolated from the same rats. The results demonstrate that LP byproducts are not only biomarkers, but potential therapeutic targets in acute SCI. Future studies will explore the mitochondrial and neuroprotective effects of compounds that block LP reactions and/or scavenge toxic LP-derived aldehydic products following SCI. Support: NIH-NINDS 1F32NS063744-01 (RAV); NIH-NIDA T32DA022738-01 (EDH); NIH-NINDS 5P30 NS051220-01 (EDH); Kentucky Spinal Cord and Head Injury Research Trust#6-5 (EDH).

P-101 Hippocampal Neuron Injury Mediated By The Innate Immune System During Acute Picornavirus Infection Of The CNS: A Paradigm For Calpain Inhibition In Neuroprotection

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Picornavirus infection is a socioeconomically important cause of morbidity and mortality in the developing world. Enteroviruses and other members of the picornavirus family maintain significant neurovirulent potential. Hippocampal injury, with consequent learning and memory deficits, is a sequela of neurovirulence that is observed in patients, especially pediatric patients. We examined the pathological and functional consequences of acute central nervous system picornavirus infection using the Theiler's murine encephalomyelitis virus (TMEV) model system. TMEV-infected C57Bl/6 mice demonstrated significant hippocampal injury at four days post-infection

(dpi), with apoptotic loss of both TMEV-infected and bystander, uninfected CA1 pyramidal neurons. In contrast, infected SJL and C57Bl/6xSJL F1 animals displayed minimal hippocampal pathology over a similar, acute time course. Functional, scent-based novel object recognition and spatial memory testing results mirrored pathological findings; TMEV-infected C57Bl/6 animals demonstrated impaired recognition and spatial memory while TMEV-infected SJL animals maintained memory function. Adoptive transfer of activated C57Bl/6 neutrophils into SJL animals resulted in hippocampal injury and abolished recognition memory function, suggesting that brain infiltrating neutrophils are sufficient to induce pathology and cognitive dysfunction. Likewise, antibody-mediated depletion of neutrophils in acutely infected C57Bl/6 mice preserved hippocampal neurons and memory function. Neutrophil-mediated, apoptotic neuronal loss was associated with the intraneuronal activation of calpains (calcium activated cystine proteases). Inhibition of calpains by BDA-410, a specific calpain antagonist with favorable in vivo pharmacokinetic and toxicity profiles, was neuroprotective in both TMEV-infected C57Bl/6 mice and neutrophil-treated hippocampal neuron cultures. Immune-mediated, CA1 hippocampal injury with corresponding functional deficit is an important aspect of picornavirus neurovirulence; calpain inhibition may offer a unique neuroprotective therapeutic strategy to preserve bystander neurons during innate immune system attack of the CNS.

P-102 Hippocampal Slice Stimulation With A Silicon Carbide Electrode Device

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It has been demonstrated that implantable brain machine interface (BMI) technology has the potential to be a therapeutic solution to assist patients suffering from damage to the central nervous system (CNS). Unfortunately, present devices are recognized by glial cells as foreign material, leading to gliosis encapsulation. Cubic silicon carbide (3C-SiC), wide band-gap semiconductor material, may provide an excellent platform for the generation of a permanently implantable planar neural prosthetic interface component of a BMI system. Previously, we have reported that this material has good flexibility, strength, and extraordinary chemical resilience to the harsh body environment. Importantly, 3C-SiC possesses superior biocompatibility when compared with cell treated polystyrene and is not encapsulated by glia. Because of these unique features, a neuronal activation device (NAD), a planar microelectrode probe, was constructed from 3C-SiC with the goal of eliciting a neuronal response in the CA1 region of hippocampal slices. The hippocampus was harvested from C57 wild type mice, cut into 400 uM slices, and the slices placed on the NAD. The NAD was perfused with heated artificial cerebral spinal fluid (ACSF). A biphasic pulse transmitted between two electrodes located under the Schaeffer's collaterals was used to elicit a response in the stratum radiatum in the CA1 region in the hippocampus. We used a Ca^{2+} sensitive fluorescent dye, Rhod-2 as an indicator of response, and stimulation was recorded using a SciMedia MiCAM Ultima L neuronal

imaging camera. A second recording method used an external glass electrode which was placed in the stratum radiatum of the CA1 region of hippocampal slices, and the subsequent field excitatory postsynaptic potentials (fEPSPs) were recorded. The results from this and our previous biocompatibility studies suggest that 3C-SiC is an excellent platform to interact with the brain as an implantable neural prosthetic.

P-103 Hypertonic Saline Attenuates Magnetic Resonance Imaging Indices Of Spinal Cord Hemorrhage And Edema After Acute Unilateral Spinal Cord Contusion Injury In Rats

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We have used magnetic resonance imaging (MRI) previously to show the subacute to chronic changes that occur after unilateral cervical spinal cord injury (SCI) in rats. Here we show the acute stage of SCI and the effect of hypertonic saline (HS) on MRI indices of swelling, hemorrhage and edema. In addition we quantified spinal cord water content (SCWC) at 6 time points after SCI to determine the time of maximal edema. 48 adult rats were subjected to unilateral SCI at C5. 12 animals were imaged for 8 hours continuously using a 4.7T MRI Bruker System. Animals were administered 0.9% NaCl (n=6) or 5% NaCl (n=6) at 1.2ml/kg IV every hour starting 30min after SCI. 42 animals were used for spinal cord tissue collection at time 0 (uninjured, BL) and at 30 min, 24hrs, 48hrs, 72hrs, and 4 and 5 days after SCI (n=6 per group). SCWC was determined in 3 6mm blocks of the cord using the wet-weight-dry-weight method. In animals that received HS, MRI indices of swelling ($p=0.017$), edema ($p<0.01$) and hemorrhage ($p=0.002$) were attenuated at 8hrs after SCI. There was an increase of SCWC at the level of the lesion over time. BL SCWC was 69.3±0.7% and SCWC increased (30min: 70.8±0.3%; 24hrs: 70.7±1.0%) to reach a maximum of 73.4±0.4% at 72hrs after SCI. This was significantly higher than at baseline, 30min and 5 days (5d: 70.5±0.6%; $p\leq0.003$). In addition, SCWC was significantly higher at 48hrs (72.2±0.1%) and 4d (72.3±0.8%) compared to BL ($p\leq0.003$). Edema formation after trauma negatively impacts blood flow and oxygen delivery to tissues. Reduction of edema and hemorrhage and spared microvasucalture may reduce secondary injury and promote repair. Functional benefit of HS in this model is under current investigation. Supported by NS-31193, NYS SCIRP CO19772, C.H. Neilsen Foundation, and the Roman Reed SCI Program.

P-104 What Is The True Contribution Of The Rubrospinal Tract To Skilled Reaching?

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Over the last decade, one fiber pathway in the spinal cord that has been the focus of much attention is the rubrospinal tract (RST) that runs in the dorsal aspect of the lateral funiculus (LF). Convergent evidence from lesion studies that include variable extents of the LF but that consistently disrupt the RST support the view that the RST subserves several components of the reach. Such lesions, however, also damage other pathways within the LF that possibly contribute to reaching. Importantly, we have recently shown that damage to dorsal roots, which can easily be created by these different spinal cord lesions, interferes with skilled reaching (Wu et al., 2009). The present study was designed to isolate further the precise contribution of the RST to skilled movements of the forelimb. Long-Evans female rats were trained to reach for single sugar pellets and were subsequently subjected to either 1) large LF lesions (LF group), 2) lesions limited to the dorsolateral aspect of the LF (DLF group) or 3) small DLF lesions that encompassed the RST (RST group). All three types of lesions included the full extent of the RST and care was taken in all instances to leave the dorsal roots intact. Detailed movement analysis revealed that, although all operated animals were still able to reach for food, the three types of lesions differently affected some components of the reach. Most interestingly, however, the pronation and arpeggio movements were impaired or missing to the same extent in all groups of lesions, suggesting that these components of the reach are under the control of the RST. The results are discussed in terms of translation of animal models of cervical spinal cord injury to clinically relevant therapeutic scenarios to improve the quality of life of people living with quadriplegia.

P-105 A Bilateral Cervical Contusion Injury Model in Mice: Assessment of Gripping Strength as a Measure of Forelimb Motor Function

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Here, we describe a bilateral cervical contusion model for mice. Adult female mice received graded bilateral contusion injuries at cervical level 5 (C5) using a commercially available impactor (the IH device). Three separate experiments were carried out to define conditions that produce impairments in forelimb function without unacceptable impairment of general health. A grip strength meter (GSM) was used to assess gripping ability as a measure of forelimb motor function; lesion size was assessed histologically by staining cross sections for H&E and GFAP. In Experiment 1, mice received injuries of 30 kilodynes (kdyn); these produced minimal deficits on grip strength. In Experiment 2, mice received injuries of 75 kdyn and 100 kdyn. Injuries of 75 kdyn produced transient deficits in gripping that recovered between 3-15 dpi to about 90% of control; injuries of 100 kdyn produced deficits that recovered to about 50% of control. In Experiment 3, none of the mice that received injuries of 100 kdyn recovered gripping ability. Histological assessment revealed graded injuries that ranged from damage limited primarily to the dorsal column (DC) to damage to the DC, grey matter, ventral column and lateral column. Fluid-filled cystic cavities were found in 13% of the 100 kdyn injury group. Whereas, a combination of fibrous-filled/fluid-filled cystic cavities were found in 22% and 38% of the 75 kdyn and 100 kdyn injury groups, respectively. There was minimal urine retention following cervical contusion injuries indicating preservation of bladder function. Our results

define conditions to produce graded bilateral cervical contusion injuries in mice and demonstrate the usefulness of the GSM for assessing forelimb motor function after cervical contusions.

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P-106 Video-Based Assessment of Motor Functions After Compression Spinal Cord Injury In Rats

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Aims and Objectives. Standard methods for motor function evaluation after spinal cord injury (SCI) in rats, for example the Basso-Beattie-Bresnahan (BBB) scale (Basso et al., J Neurotrauma 12, 1995, 1), are semi-quantitative and estimate different aspects of locomotion, such as plantar stepping, limb coordination and trunk stability, by a single score. Here we tested the potential of a novel numerical approach described for mice (single-frame motion analysis, Apostolova et al., J Neurosci 26, 2006, 7849) in a rat compression SCI model. *Materials and Methods.* Young adult female Wistar rats (n = 10) were subjected to a moderate spinal cord compression at T8 level using fine forceps driven by a custom-made electro-mechanic device. Prior to operation and 1-9 weeks thereafter, the rats were video recorded during unforced beam walking and inclined ladder climbing. Using selected video frames, three parameters were measured: foot-stepping angle (FSA) and rump-height index (RHI) during beam walking and number of correct steps during ladder climbing. *Results and Discussion.* One week after SCI, the rats developed severe disabilities. Compared with intact animals, the FSA was increased by a factor of 8, the RHI decreased two-fold and the number of correct steps with the hind limbs on the ladder declined from 6 to 0. All three parameters improved during the 9-week recovery period, however, to markedly different degrees. The FSA, an estimate of the plantar stepping ability, recovered to nearly 100% of control. The RHI, a measure of the ability for coordinated limb movements and body weight support, improved to 70% of normal. The number of correct steps, a parameter dependant on central connectivity and proprioception, recovered to only 28%. *Conclusion.* Our findings indicate the potential of the novel approach for precise evaluation of motor abilities requiring different levels of spinal and supra-spinal control after SCI in rats.

P-107 Adult Male And Female Wistar Rats Differ In How They Locomote

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INTRODUCTION: Various biological factors, such as the strain or breed of an animal, and the age of an animal, contribute to behavioural differences of animals (Webb et al, 2003; Webb et al, 2007). In the last decade, research addressing behavioural differences between sexes of animals has increased considerably. Sex differences in rats' behaviours have been addressed with the consensus that there are important differences between females and males. Since rats are a popular species of study in the neurosciences, it is necessary to identify factors that may affect the outcome of a behavioural task. This is especially important when assessing functional recovery after injury to the peripheral (PNS) or central nervous systems (CNS). Amongst the different types of behaviours that have been examined in female and male rats, an area that has been under-examined is locomotion. **METHODS:** Seven 2-month old adult female, and 7, 2-month old male Wistar rats were examined. Sensorimotor, especially locomotor abilities were measured for all rats. Specifically endpoint (tapered beam, ladder rung-walking, paw preference, inclined plane, BBB locomotor rating scale, BBB-subscore rating scale), kinematic (locomotion while trotting), and kinetic (ground reaction forces while trotting) measures were made. **RESULTS:** Though no sex differences were found for sensorimotor endpoint measures, sex differences for locomotion were revealed using biomechanical analyses. In particular, male rats bear more weight with their hindlimbs compared to females during trotting. Also, males and females differ in the range of motion of their hindlimb joint angles during locomotion. **CONCLUSIONS:** These results suggest that sex should be considered when designing experiments and when comparing data from within or between labs. **SUPPORT:** NSERC grant to AAW; Queen Elizabeth II Scholarship to SCKN

P-108 Induction And Detection Of GDNF As An Axonal Attractant

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Spinal cord injury results in loss of tissue and function at and below the injury site. We have developed a human embryonic stem cell-derived motor neuron cell replacement strategy to replace lost motor neurons at an injury site with new cells that may establish new connections with the host. Enhanced GDNF secretion in the periphery has been suggested as an effective strategy to promote motor neuron axon outgrowth. To this end, we developed a lentiviral GDNF construct and established detection methods of GDNF *in vitro* and *in vivo*. We then developed a bioassay for GDNF and measured PC12 neurite outgrowth to determine GDNF effects on motor neuron progenitors in vitro, and found that GDNF induced significant outgrowth. Here, we report methods to detect GDNF i*n vivo* after myoblast transplantation and discuss relevant transduction and transplantation procedures and their affects on GDNF expression. Three main methods are being used to detect GDNF production both in vitro and in vivo; 1) a real time PCR assay was developed to measure GDNF gene expression, relative to housekeeping gene GAPDH, 2) immunostaining of

human nuclei and GDNF secretion showed localization of GDNF to the transduced cells, and 3) a GDNF ELISA was used to detect the secreted protein in conditioned medium, transduced cell extracts and muscle extracts. Results from all in vivo and in vitro techniques showed a decrease of GDNF over time. Additionally, the immunostaining showed transplant cell survival and localized protein secretion from the injected muscle and cell extracts. In conclusion, we have established multiple assays to detect and confirm GDNF secretion in vitro and in vivo, which will permit optimization of GDNF expression levels for future studies and therapies.

P-109 Industry Standard Manufacturing of Medias, Supplements, And Transplant Solution For Neural Regeneration Research

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The development of clinical grade, animal-free media formulations, supplements and buffers is important for the preparation of cellular material that is intended for use in humans. We have developed and tested an array of products relevant to the propagation of human embryonic stem cells (hESC) and hESC derivates. Our media line consists of StemBlast, MotorBlast, and NeuroBlast media formulations; our supplement line currently consists of CSC Neural Supplement; and our cell buffer line currently consists of Cell Transplant Solution (CTS). StemBlast is a fully defined, serum-free, feeder free hESC propagation media with an osmolarity and pH optimized for human cellular physiology. StemBlast was compared with the leading commercially available stem cell media products and found to be equally equipped for propagating hESCs while retaining their pluripotency for over 20 passages. MotorBlast is designed for the complete growth, maintenance differentiation and maturation of human motor neurons (MNs) and motor neuron progenitors (MNPs) derived from hESCs. Using MotorBlast and NeuroBlast medias, with the addition of CSC Neural Supplement we have been able to produce clinically relevant quantities of pure neuronal progenitors (NPs), and MNPs, which are capable of becoming fully functional after maturation using these media products. Our CTS is made completely of USP grade components and is designed to be used as a human injectable solution or clinical grade washing or transplant buffer solution. When compared to standard growth media at multiple time points, CTS significantly improves the viability of the cells. In addition, use of CTS for *in vivo* transplantation results in enhanced graft survival, demonstrating that CTS is a cellular vehicle of choice. Therefore, these products are adequate in enabling neural regeneration research to be taken from the bench to the clinic.

P-110 Methods For Improving Localization Of Deep Brain Implants

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Both electronic neuroprostheses and cell-based central nervous system implants must be precisely placed for best efficacy, ideally with < +/-0.5 mm error. Both types of treatment utilize the same stereotactic neurosurgery hardware and software. Methods for high-precision placement of Deep Brain Stimulation (DBS) electrodes are transferable to surgical implantation of stem cells to correct motor and neuropsychiatric disorders.

The sequence of events in DBS surgery begins with magnetic resonance (MRI) and computerized tomography (CT) imaging with voxel dimensions of 1 - 2 mm. Measures are taken to reduce motion artifact in patients with tremor or dystonia. Commercial planning software operates on MRI and CT images to identify targets and safe trajectories from entry points on the skull, aided by reference to a scalable anatomic atlas and recently by Diffusion Tensor Imaging (DTI). Either frameless or frame-based stereotactic apparatus is fixed on the patient's head and set for the selected trajectory; with care, optically tracked frameless devices can achieve the accuracy of older headring and arc systems. Intraoperative biaxial plane X-rays yield the best available measurement of deviation from planned trajectories of microelectrodes for neuron firing pattern mapping and subsequent permanent electrodes or cellular implants. Contrast ventriculography provides co-registration of intraoperative X-rays with pre-op MRI within +/-0.3 mm. Custom software allows determination of electrode or cell-injection cannula tip location in anatomic (Talairach), "surgeon's eye" and stereotactic coordinate systems.
Cell-based implants have localization advantages over DBS electrodes, which need to be rigidly capped at the skull (with risk of electrode movement) and cause artifacts on post-op MRIs. However, cells and their carriers or scaffolds are not inherently visible in post-op imaging, so intraoperative localization is the only verification of correct placement. Consequences of misplacement by >2 mm include stimulation of unintended targets and decreased battery life or functional cell population.

P-111 Dispelling A Misconception: Radiation Therapy Per Published Data Is A Curative Procedure For Spinal Cord Injury Rather Than Lethal

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Most papers on pre-clinical studies in spinal cord (SC) injury maintain the dogma that "no effective therapeutic interventions exist for severe spinal cord injury." While currently true for human injury, in the pre-clinical setting, published data show that radiation therapy is an effective curative procedure for complete transection[1-2] and severe crush SC injury[3-4]. Nevertheless, these data are ignored by investigators who concurrently also perpetuate misconceptions that radiotherapy is harmful/lethal; consequently the translation of radiotherapy as a treatment for human injury is hampered/prevented. Radiotherapy is a life-saving clinical modality used specifically to eradicate solid tumors. Radiotherapy of the SC-injury site can facilitate restoration of structure[1,3] and of brain-controlled motor function[2] below the lesion. It eradicates cells that interfere with natural repair following injury[1]. Furthermore, combining radiotherapy with training in severe contusion[4] resulted in significant restoration of standing and stepping capacities. Nevertheless, a grant proposal aimed at improving

training protocols for 'teaching the repaired SC to walk' was rejected because 'radiotherapy is lethal', citing the study in which the animals died indiscriminately[5], not due to irradiation but due to negligent care. Thus, the ISNR will serve as a forum to differentiate the scientifically valid from the superstition.

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